Biochemical markers of health for children with poor anthropometric status in urban Nepal

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BIOCHEMICAL MARKERS OF HEALTH FOR CHILDREN WITH POOR ANTHROPOMETRIC STATUS IN URBAN NEPAL

A Thesis for Master of Philosophy

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To the children and mothers in Pathibhara and Balaju,

My friend Laxmi Sharma and her father,

The Thapaliyas - my Nepalese host family,

And my mum and dad

Acknowledgements

I would like to give sincere thanks to my supervisor, Dr. Catherine Panter-Brick for her huge help with my research and her patience with my poor English. I would also like to thank Dr. Shyam Thapa at the Family Health International and Dr. David Osrin at the Mother and Infant Research Activities (MIRA) in Nepal for their help with preparation and advice for the research invaluably. Dr. Thomas McDade at the Northwestern University in US, Dr. Christine Northrop-Clewes & Dr. Anne Susan Mburu at the University of Ulster in Northern Ireland, Dr. Renu Manandhar and Dr. Nhuchhe R. Tuladhar in the Tribhuban University in Nepal helped with sample analyses. Mr. Giri Manandhar in Simca Ltd. and Dr. Tuhin Bhattacharya donated much medication for the children. Mrs. Lajana Manandhar and Miss. Kumari Tandukar in Lumanti helped to introduce my study to the communities. Dr. Buddhi Man Shrestha and Dr. Bill Simon offered the liquid nitrogen. Dr. Robert Drewett kindly supported statistical analysis. Without the help of these people, this study would not have been possible. So I would once again like to warmly extend my thanks to them all.

ABSTRACT

This study examines the relationship between poor anthropometric status and biochemical markers in children in urban poor Nepal. To date, no study has examined poor nutritional status in the urban poor children in relation to biochemical markers to explain the conditions of children's health in polluted squatter environments, nor considered possible associations with weaning practices. In a cross-sectional study, 0-60 month old squatter children were examined anthropometric status, intestinal mucosal function (intestinal permeability), clinical/sub-clinical inflammation status (acute-phase protein) and parasitic infection. 210 children were measured for height (or length) and weight, 167 for intestinal permeability (lactulose/mannitol ratio: L/M ratio) and 173 for acute-phase protein (C-reactive protein: CRP) and parasitic infection. Data on weaning practices and morbidity were collected from 172 mothers/guardians. No significant relationships were found between anthropometric status per se and the levels of L/M ratio or CRP. The L/M ratio (0.26) was poorer than that of UK children (0.12) but similar to that found in studies of children in Bangladesh (0.24). Giardia cases (N=8) had worse L/M ratios than those non-infected (0.43 versus 0.25 respectively, p=0.014). A measure of weaning practices, a longer period of lactation, was associated with poorer intestinal permeability (p=0.031), as well as with poorer height-for-age (p=0.024) in children who had ceased breastfeeding. Also, the maternal morbidity reports reflected the current health status of children: high CRP values, reflecting acute-inflammation status, were associated with reported illness on the day of interview (p=0.004) but not with the preceding 7 days' reports. This study showed the importance of weaning practices as a two-stage process - the onset of supplementation and the cessation of breastfeeding. These two stages may have a differential impact on children's health in early life stage and poor nutritional status. Maternal report was useful as a proxy for child morbidity, sensitively reflecting children's acute illness in urban Nepal.

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INTRODUCTION

1.1. Poor Anthropometric Status of Children in Urban Nepal

South Asian countries carry the worst burden of child malnutrition in the world. Indeed, in Nepal, the assessment of anthropometic status among 6-36 month-old children has shown that 49% are underweight and 64% are stunted (Nepal Multiple Indicator Surveillance, 1995; in UNICEF, 1996). The poor nutritional status leads to the risk of high mortality and morbidity. In Nepal, 75 in 1000 live birth infants died before reaching one year of age and 104 children died under 5 years old (UNICEF homepage, 2001). Nepal has one of the highest percentages of child population in the world: those under 15 years of age represent 42% of the total population and those under one and five years of age represent 4% and 16% of the total population in 1991 respectively (by Central Bureau of Statistics; in Adhikari and Krantz, 1997). Therefore, the problem of malnutrition among Nepali children is very important issue of concern for the country.

Nepal is categorised by the United Nations as one of the least developed among the Third World countries and about half of the population is considered to exist below the absolute poverty level. The basic causes of malnutrition in the Third World often lie in poor socioeconomic conditions. Vice versa, poor nutritional status of children is an indicator of inappropriate living conditions and a poor environment (Tanner, 1986; Panter-Brick, 1998). Poor anthropometric status in Nepal is very common, not only in the rural population (Costello, 1989; Panter-Brick, 1997), but also among the urban poor (Panter-

Brick et al., 1996; Moffat, 1998). As is the case in other developing countries, the absolute number of urban poor is increasing. Because the country is in the early expanding stage of the demographic transition characterized by high birth rates and declining death rates, it has experienced a rapid population growth over the last four decades. Thus, the population growth and prevailing poverty in the villages have provoked a rural-urban migration in search of better life, with the hopes of securing jobs and chances of higher education for children as well as better health supports. The accelerated urban economic activities and the increasing migration from villages change the environment of urban areas very rapidly. The decade 1971 to 1981 was one of rapid population growth for urban areas: the urban population had reached 958,000 inhabitants in 1981, increasing by over 69% in one decade. Furthermore, it reached 1,690,000 by 1991, increasing by over 46% within another decade (National Habitat II Committee, 1996).

This urban growth has been very rapid, but the social and economic infrastructures are already inadequate and the rapid rural-urban migration puts an excessive pressure on limited urban resources and social services. The size of the urban poor was roughly estimated at about 63,000 by the government (National Habitat II Committee, 1996). The urban homeless population is also increasing, due to lack of housing in the urban areas as well as rents being high for available housing, therefore, making it almost impossible for migrants from villages to own property.

Gallagher (1992) mentioned squatting issues in urban Nepal. The author defined squatting as 'the occupation of land to which one possesses no *Lal PurJa* (land ownership certification), or in the case of renters, the occupation of land, housing or rooms to which the landlord possesses no ownership certificate (Gallagher, 1992:249)'. Those in illegal settlements were estimated in Kathmandu as 8,000-10,000 people (Gallagher, 1992). The urban infrastructure facilities are too inadequate to cover the demands of the increased poor population. Particularly, sanitation problems in such squatter areas are usually the worst and it easily harms the people's health, particularly children's, in these areas.

1.2. What Are the Reasons for Malnutrition?

The delayed maturation in children from developing countries may be related to the complex effects of malnutrition, malabsorption and infection early on in life, particularly between the ages of 3 and 12 months old (Martorell and Ho, 1982; Lunn, 2000). Indeed, Nepali children show generally adequate growth during early infancy. However, growth faltering is evident from 6 to 12 months (Costello, 1989; Moffat, 1998). The poor growth in the Third World is a result of poverty and huge burden of infectious illnesses brought about by poor hygiene and sanitation using contaminated water supplies and ignorance regarding correct infant feeding practices.

1.2.1. Unsanitary Condition and Poor Growth

Solomon et al. (1993) focused on the relationship between an unsanitary environment and poor child growth offering an unique viewpoint: "a dirty chicken is poorly growing child (Solomon et al., 1993:328)". The authors gave an example from veterinary practices: chicks reared under sanitary and hygienic conditions showed high rates of growth, and adding antibiotic to their feed did not promote additional growth. In the following experiment chicks raised in unsanitary conditions gained less weight, but adding antibiotics to the feed allowed the birds to attain growth rates similar to those of chicks in the sanitary environment. It may be possible to apply this example to humans in poor hygienic circumstances in developing countries, thereby highlighting the important relationship between the infection burden in an unsanitary environment and early child growth.

1.2.2. The Timing of Weaning and Poor Nutritional Status

The timing of weaning, beginning with the introduction of supplementary foods, is a particularly important point in a child's development. However, supplementary foods were often contaminated by diarrhoeal pathogens (e.g. *E. coli*, rota virus, dysentery bacillus), or soil/water-transmitted parasites through contaminated foods and utensils introduced those infections to young babies which may damage the small intestinal mucosa. McDade and Worthman (1998) noted that the determinants of introduction of supplementary food and exclusive breastfeeding are a complex short- and long-term trade-off of infant needs and constrains activity in the variability of biosocial and cultural aspects described as the "weanling's dilemma", especially for infants after 6 months of age. At this time breastfeeding alone becomes insufficient for nutrition requirements and maintenance of body function and growth for children, whereas starting dietary supplementation, on the other hand, raises the risk of infection and so may cause diarrhoeal illness and malnutrition.

The timing of growth faltering may relate to the point of the introduction of supplemental foods. For example, the growth faltering of Gambian infants occurs early on in life. While the infants' length and weight in the first 3 months of life are parallel to the 50th centile, the growth beyond 14 months approaches the 5th centile, although no catch-up growth during the second year of life is observed (Lunn et al., 1991b). The authors mentioned that chronic diarrhoea was a major factor in the growth faltering in the Gambia and suggested that impaired gut function in early life due to intestinal infection is a serious trigger of malnutrition in children.

1.2.3. Food Malabsorption and Poor Growth

Lunn (2000) suggested that food malabsorption is a significant cause of malnutrition, resulting from impaired function of the small intestinal mucosa. The Gambian infants showed a markedly poorer intestinal permeability (to indicate the condition of food absorption) than UK healthy children (Lunn et al., 1991a) and their growth velocity was negatively associated with impaired intestinal permeability. The authors showed that the levels of intestinal permeability predicted 43% for length growth and 39% for weight growth in the study. Even levels of intestinal permeability have been related to feeding practices. In Guatemala, Goto et al. (1999) reported on a correlation between intestinal permeability and the time of weaning. The cessation of breastfeeding at an early age worsened intestinal permeability values for infants 0-11 months of age. There was no significant correlation between the duration of the non-breastfeeding period and the levels of intestinal permeability. These results suggested that the early termination of breastfeeding rather than the amount of time since weaning would be associated with altered small intestinal mucosal function.

Lunn (2000) outlined two possible mechanisms leading to undernutrition (Lunn, 2000:148). First, following infection by dietary pathogens, possibly from unhygienic food preparation or storage, the villi in small intestinal mucosa are damaged and lose the vulnerable disaccharide lactase, leading to maldigestion of lactose and probably other nutrients. Second, the barrier function in the small intestinal mucosa is compromised by pathogens, allowing translocation of macromolecules, resulting in mucosal inflammatory and immune reactions and associated impaired growth of children. The association between growth and gut damage is potentially of great importance when determining the mechanisms underlying child malnutrition and ill-health in the Third World. It is therefore important to test whether such associations hold in other populations where unsanitary conditions and growth stunting are extremely common problems. Mucosal function can be assessed using the dual sugar intestinal permeability test, which is used in the clinical investigation of small bowel diseases (Lunn et al., 1991b) but is also valuable as a non-invasive test of gut function at the community level.

1.2.4. Parasite Infections and Poor Growth

The association between growth retardation and parasite infection is, however, not fully understood. Previous case-control studies have shown conflicting results: no impact on growth (Greenberg et al., 1981; Rousham and Mascie-Taylor, 1994), significant impact on growth (Stephenson et al., 1989; 1993; Stoltzfus et al., 1997) or uncertain outcome on growth (Freij et al., 1979; Hadju et al., 1997; Raj et al., 1998). Hall (1993) subsequently mentioned several differences in each study, regarding the environments of children or the intensity and the duration of infection, which may explain the results of growth improvement found in the case-controlled studies. Other studies have suggested that the impact of re-infection (Greenberg et al., 1981; Stephenson et al., 1993), age factors (younger children showed better growth) or children's initial growth status (less stunted children showed better growth) (Stoltzfus et al., 1997) could produce a different impact on growth. One study, by Northrop-Clewes et al. (2001) in northern Bangladesh, implicated the type of parasite. It compared the difference of z-scores in bimonthly measurements on growth between the anti-helmintic treatment (for Ascaris, Trichuris and hookworm) and placebo groups. It was, however, found that Giardia infection and weight loss showed an association.

Impact of parasitic infection on intestinal permeability is not clear. Levels of intestinal permeability before and after the treatment of Ascaris infection have been investigated, but significant differences in the intestinal permeability are not obvious (Northrop et al., 1987). Furthermore, the intervention study in rural Bangladesh using a wide spectrum of anti-helmintic treatment showed no changes of intestinal permeability between treatment and placebo groups (Northrop-Clewes et al., 2001). However, children who were infected by Giardia were observed to have higher intestinal permeability than other children in the study. Thus, Lunn et al. (1998) found a positive association between the level of Giardia-specific IgM antibody and intestinal permeability. Therefore, the relationship between parasitic infection and intestinal permeability was unequivocal, though protozoa such as Giardia may have a critical impact on intestinal mucosal function.

1.2.5. Clinical and Sub Clinical Inflammation Status, Morbidity and Growth

Acute-phase proteins such as acute inflammation markers can reflect not only clinical, but also sub-clinical inflammation status. Some studies reported the relationship between poorer growth status in the Third World countries and high infection status from examining levels of acute-phase proteins using such as C-reactive protein (CRP), a 'short life' acute-phase protein, and α_1-antichymotrypsin (ACT), a 'long life' acute-phase protein. Examining the relation of infection to child growth status in Nepal, Panter-Brick et al. (2001) reported a significant association between growth stunting and very high levels of ACT in 10 to 14 year-old Nepali boys sampled from both village and urban populations. Similarly, Parkin and Lunn (1998) reported that higher levels of ACT were related to poorer anthropometric status on height-for-age and weight-for-age z-scores in 5-12 year-old Nepali rural children. In the Gambia, Lunn et al. (1998) examined the relationship between infection burden and severe growth faltering and found that elevated levels of ACT were related to the poor weight gain of infants between 2 to 8 months of age. In Zambia, Hautvast et al. (2000) examined the relationship of infections with subsequent 3-month length measurement in infants and also found that that the levels of CRP were weakly, but significantly, correlated with 3-month length increments.

With respect to associations between acute-phase protein and morbidity, few studies have focused on Third World communities, though their results often show high agreement between the variables. Thus, elevated levels of CRP was significantly associated with fever (in Zambian children 2-20 months old; Hautvast et al., 2000), with vomiting (in South African children 1-5 years old; Filteau et al., 1995) and with observed respiratory symptoms (in Samoan youth 4-20 years old; McDade et al., 2000). Elevated ACT levels were significantly associated with diarrhoea and fever (in Nepali 5-12 years old children; Parkin and Lunn, 1998), with fever and the combination of fever/respiratory infections, and diarrhoea (in Bangladeshi children 2-5 years old; Rousham et al., 1998).

1.3. Objectives of the Study

The studies previously mentioned have been concerned with exploring the reasons for poor nutritional status in the Third World countries and correlating anthropometric status with biochemical markers. To date, no study has examined anthropometric status in the squatter communities of Nepal in relation to biochemical markers to explain the condition of children's health in polluted urban environments, nor considered possible associations with weaning practices.

The present cross-sectional study examines the relationship between anthropometric status, intestinal permeability, levels of acute-phase protein, parasitic infection, reported morbidity as well as weaning practices in Nepali children 0-60 months old. It focuses on children in poor urban squatter areas where poor socio-economic status and levels of sanitation are the rule.

The objectives of the study are:

1. to document anthropometric status in squatter areas, for children 0-60 months old, showing the timing of growth faltering and anthropometric status around weaning
2. to measure the levels of intestinal permeability
3. to investigate whether levels of acute-phase protein are associated with morbidity reports by mothers/guardians
4. to examine relative impact of levels of intestinal permeability and acute-phase protein, morbidity reports and parasitic infection on anthropometric status
5. to evaluate how weaning practices, in both the timing of supplementation and the cessation of breastfeeding impact on levels of intestinal permeability and anthropometric status

This thesis is composed of the following chapters. This chapter is an introduction to the environment surrounding malnourished poor urban children in Nepal and a review of previous studies concerned with malnutrition among children in the Third World. Chapter 2 describes the squatter areas of the present study, the population and living conditions based on a house-to-house demographic survey. Chapter 3 details the study design, sampling and methods employed in this study. Chapter 4 shows the results of the research, with tables and figures. Finally, Chapter 5 discusses and makes conclusions on the circumstances of the squatter children.

CHAPTER 2

STUDY AREA – SQUATTER SETTLEMENTS IN URBAN NEPAL

2.1. Location, Timing and Introduction of the Study

The Kingdom of Nepal is a small, rectangular shaped country embracing the highest mountains in the world, the Himalayan range. In the north, the length is 885 km (East-West) and the non-uniform mean width is 193 km (North-South). It is bordered by India to the east, south and west and by Tibet and the Republic of China to the north. It lies in the subtropics, between 26°22' N to 30°27' latitude and 80°4' E to 88°12' E longitude in the northern hemisphere. However, a diversified climate is formed due to the mountainous landscape.

Kathmandu, the capital of Nepal, is located 1,370m above sea level in the Kathmandu Valley. The temperature is about 0℃ minimum in winter, but snow is rare, and about 30℃ maximum in summer. June to September is the monsoon season, with rain of about 200-360mm a month. It is the governmental and commercial centre of Nepal, located on an ancient trade and pilgrim route from India to Tibet or China.

This cross-sectional study was conducted from September 1999 to April 2000, during the dry season and the middle of the time was winter. I was introduced to two squatter communities in Kathmandu, Pathibara and Balaju, by a local non-governmental organization group 'Lumanti'. Activities of Lumanti for squatter people aim to help and encourage people to be self-supporting, with activities such as organizing financial or women's groups and educating people on the requirements for good health while maintaining sanitary conditions. A health worker in Lumanti introduced the present study to families as an opportunity for health checks for young children and mothers.

The two sites were chosen for the study because there were sufficient children to reach an adequate sample size, the number required for a statistical study being 215 children for growth status (see 3.7. Sample Sizes Calculation, p.32), according to the existing data from Lumanti's previous survey. Moreover, the location of sites was convenient to get public transportation and bring samples within 30 to 60 minutes of collection to Tribhuban University Teaching Hospital (TUTH), which was located almost in the middle of the two sites; those study sites were located near the Ring Road, a main route in Kathmandu (Figure 2-1).

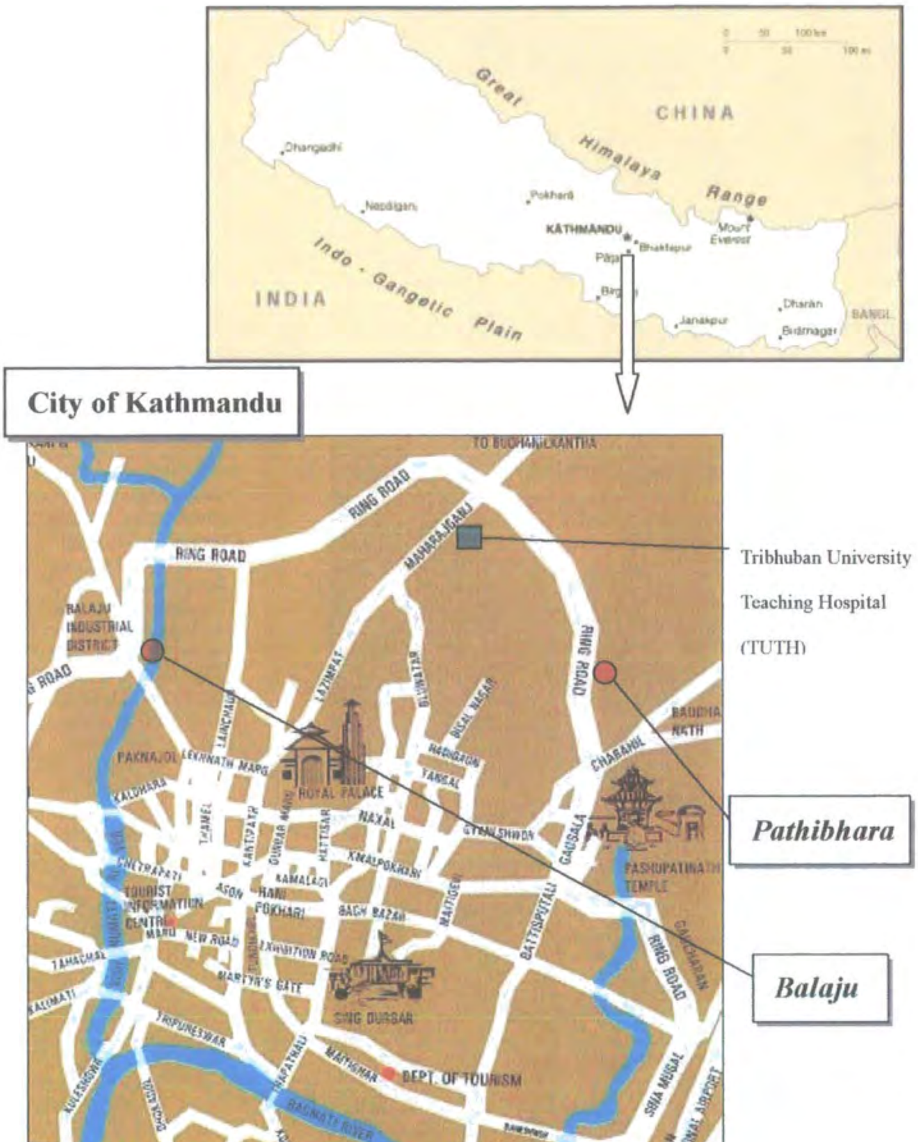


Figure 2-1. Location of Pathibhara and Balaju in Kathmandu city

2.2. '*Sukumbashi*' - The Squatter Community and People

The term '*sukumbashi*' is used for these squatter people and communities in Nepal. The *sukumbashi* generally build up simple settlements in public places such as riversides with no land ownership rights or permission to settle in the places from the government (i.e. they are illegal occupants of the land). Thus, there are few governmental supports or social welfare facilities for these people. Traditional family support also tends to be limited because it is usually the nuclear family which has become isolated from its original roots.

Both communities, Pathibhara and Balaju, suffered from very poor sanitation and low socio-economic conditions. Both research sites are situated by polluted rivers and people use the rivers for domestic activities such as washing and traveling (Figure 2-2). There are pump water facilities inside the communities and people use these for cooking and drinking.

Regarding the location of the two sites, Balaju is located on higher ground while Pathibhara, situated lower on the riverbank, is more susceptible to the risk of flooding. The squatter community Balaju is located near Balaju Industrial District and New Bus Park. Therefore, it is an entrance from the Turishuli area and many people seeking jobs and houses in Kathmandu from the mountain side, such as the Tibeto-Burmese population, settle in this area. The community is one of oldest squatter communities in Kathmandu, started about 14 years ago. Pathibhara is, on the other hand, a new community started only 4 years ago.

Figure 2-2. Polluted river surrounding community (Pathibhara)

2.3. Demographic Survey

I undertook a house-to-house demographic survey of the areas with a female field assistant for 2 weeks in each community (Pathibhara, 1st - 15th November 1999; Balaju, 28th January - 12th February 2000), reaching 95% of all households targeted. Information on family members, name, age, sex, relationships, caste/ethnicity, religion, income, education, occupation, together with housing and living conditions was obtained.

A total of 315 households and 1,411 people were surveyed in the two communities (male 727, female 674). There were a large number of children in the communities: children 0-4 years old represented 14% of the total population and 39% of the population were under 15 years old. (Figure 2-3). The size of households was not large, with a mean of 4.4 persons per household (Figure 2-4), many of them being nuclear families. In the squatter areas, people of different castes and ethnicity lived together in one place, 23 different caste and ethnic groups being represented in the communities. People could be grouped into three main categories: Tibeto-Burmese group (Rai, Lama, Tamang, Limbu, Gurung, Magar, Sherpa, Bhote), Indo-Aryan high caste (Brahman, Chettri and Newari) and low caste (Damai, Kami, Tharu, Sudra, Marji, Kajhi, Bhujel, Shahi, Puri, Jogi). More than one half of the people were in the Tibeto-Burmese group and the rest were of Indo-Aryan origin belonging to high (24%) and low (14%) caste (Figure 2-5). There were also Indians and Muslims, but these constituted only 1%. More than one half of the people were Hindu, followed by Buddhist (28%), Christian (15%) and Muslim (1%) (Figure 2-6). There was a relatively high proportion of Christians in the squatter communities; less than 2% of the people in Nepal are Christian (Costello, 1998). This is perhaps because those poor communities in urban areas are contacted by many Christian foreign groups to help those poor people.

The average monthly income per household was 4,434 Nepali Rupee (Rs), about 51 US dollars (1USD = Rs 87, in April 2000), but there was a large range of income, from Rs 500-24,000 (USD 6-276). The household income depends upon the household size, therefore, monthly income per person (monthly income in a household divided by the household size) was calculated. Many people suffered from poverty with low income: 60% of people had less than Rs 1,000 per month (USD 12) (Figure 2-7).

Access to electricity and types of roof are good indicators of poverty in Nepal. 31% of the households did not have electricity in their houses. 78% of houses had tin roofs, 12% had plastic and 10% had straw roofs. 93% of households used kerosene for cooking because of lack of firewood in the city.

61% of those over 16 year had the ability at least to read the newspaper and write the Nepali alphabet. The remaining 39% were illiterate, including 71% of women. Thus, women have less opportunity for learning. 26% of the over 16 year-olds had no occupation. Employed people in the communities worked as handcraft workers (16%), such as weavers or carpet makers for carpet making industries or souvenir makers for tourist markets. Handcrafting at home is popular job for women who need to take care of children and house works. 15% were day labourers including both men and women. Some people worked as drivers (9%), shop workers (9%), builders (7%), technicians (7%) or servants (6%). 5% of people owned or rented some pigs or ducks for meats and kept these behind their houses in small spaces and unsanitary conditions.

All children from 0-60 months old in the communities were recruited before starting the measurements. This was because, weaning is actually a long-term process during childhood. Therefore, 0-60 months were an appropriate age range to consider the impact of weaning practices on anthropometric status.

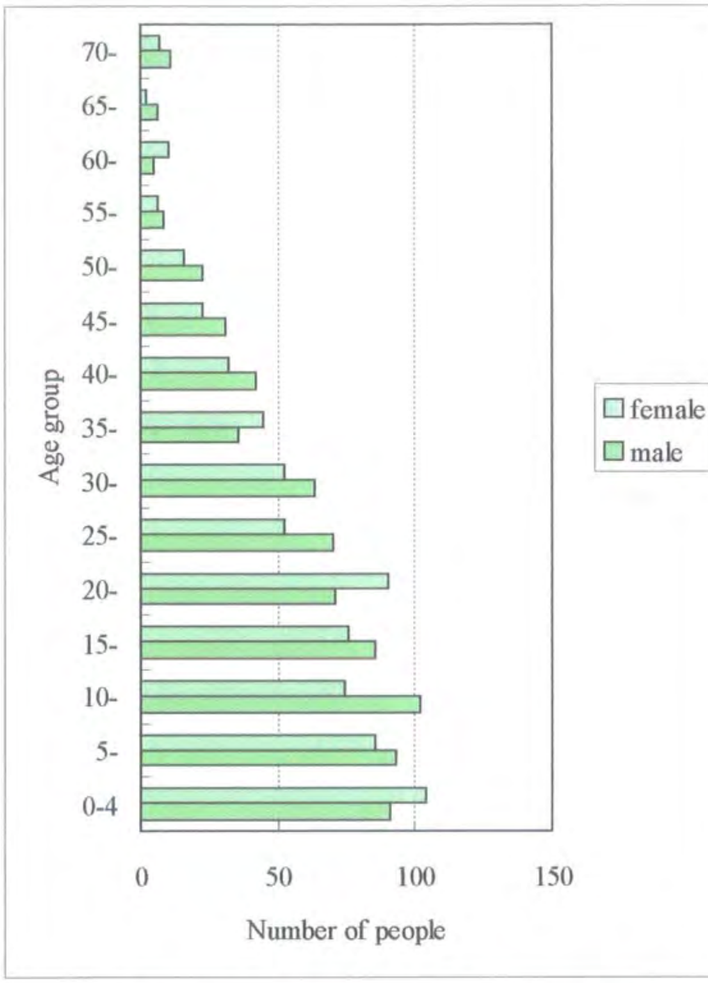


Figure 2-3. Population in the squatter areas

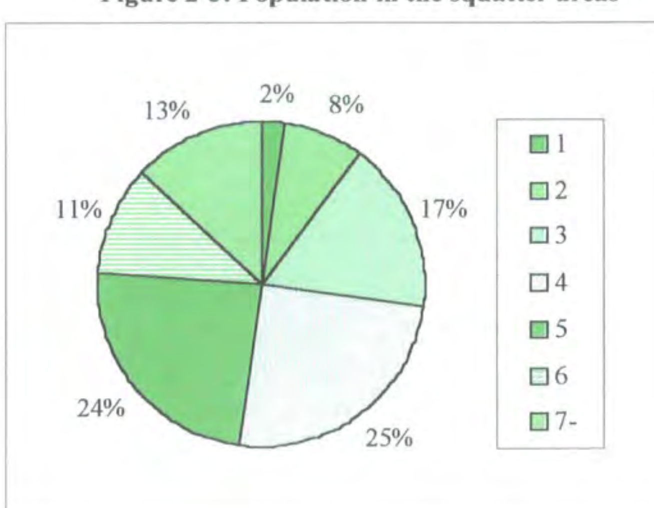


Figure 2-4. Household size in the squatter areas

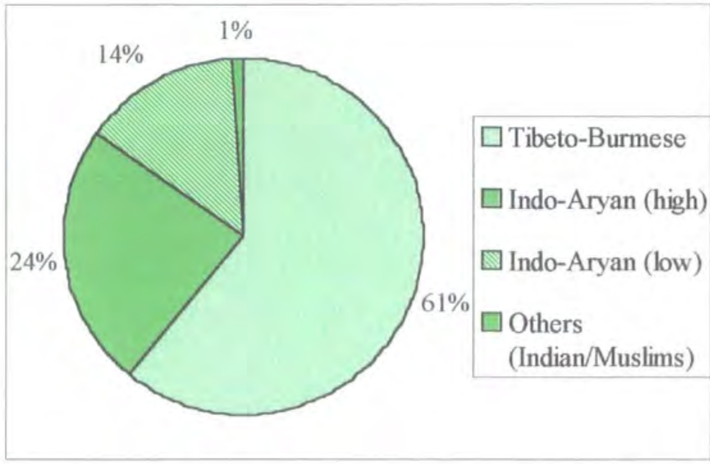


Figure 2-5. Composition of ethnicity and caste in the squatter areas

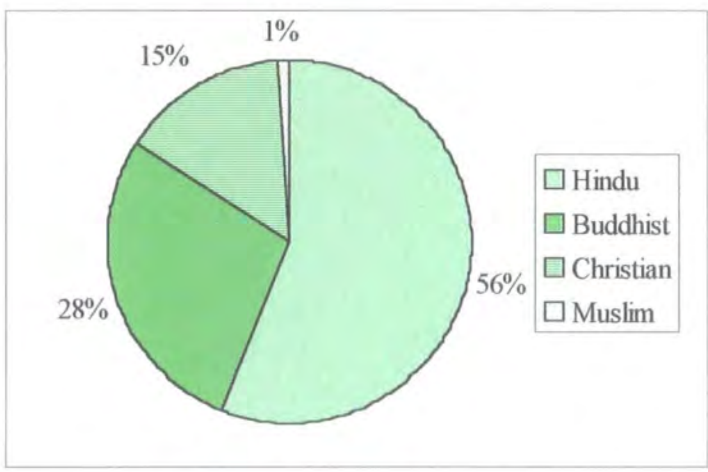


Figure 2-6. Religion in the squatter areas

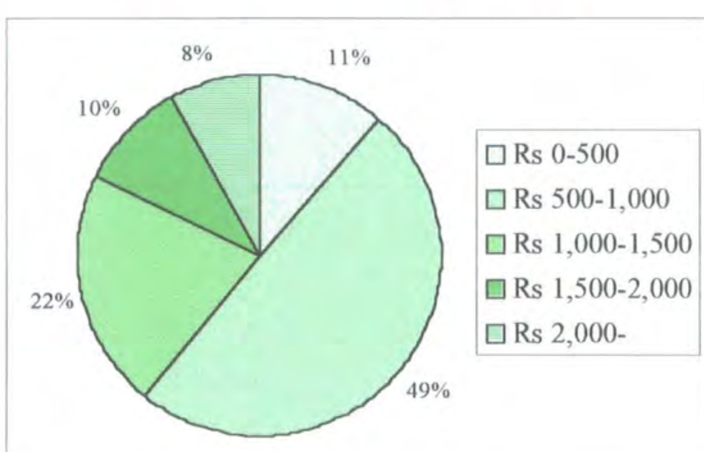


Figure 2-7. Monthly income per person in the squatter areas

CHAPTER 3

METHODS AND SAMPLES

3.1. Overview

This chapter outlines methods and sampling of anthropometry, urine collection for intestinal permeability, blood spots for C-reactive protein, stool sampling for parasitic infection and interviewing to obtain information on morbidity and weaning practices. The study was cross-sectional and the methods and sampling are considered to be field-friendly and non-invasive, particularly for children. Calculation of sample sizes aims to find out an appropriate sample size to detect the relationships between anthropometric status and biochemical markers, using the data on levels of anthropometric status and biochemical markers from the literature. Statistical analysis aims to unravel the complex relationships between anthropometric status and health condition, such as gut condition and infection status as well as weaning practices, considering the background information, age, sex, ethnicity and location of the children.

3.2. Anthropometric Status

3.2.1. Z-scores: Height-for-Age and Weight-for-Age

All the anthropometric data, children's height (or length) and weight, were analysed using two kinds of z-scores. Z-score is a standard deviation from the median of the reference population. Two z-scores, height-for-age (HAZ) and weight-for-age (WAZ), were used for calculation of anthropometric status in this study. Two types of malnutrition can be described with the z-scores: "wasting", indicating low WAZ (under

–2) related to acute malnutrition; and "stunting", indicating by low HAZ (under –2) related to chronic malnutrition.

The base data of the reference information of anthropometric status were produced by The National Center for Health Statistics (now a part of the Center of Diseases Control and Prevention (CDC), USA) in 1977. The growth chart based on these data is widely used as a clinical and research tool to assess the nutritional status and general health and well-being of infants, children and adolescents. It has been adopted by the World Health Organization (WHO) to facilitate international comparisons of growth data.

Z-scores are obtained using nutritional analysis software EPI-NUT in EPI-INFO provided by CDC. Incidentally, CDC published a new growth chart on 30th May 2000. These data were compiled to better take into account racial/ethnic diversity, and differences between breast-fed and bottle-fed infants in the United States. Unfortunately, however, CDC has not yet updated the software EPI-NUT. Therefore, I used the latest available version (version 6.03, 1996) for this study.

3.2.2. Technique of Anthropometric Measurements

Weight and height/length (for children over or below 24 months old) were measured following standard techniques (Lohman et al., 1988) using portable equipment: stadiometer (0.1 cm precision) for height or portable length board (0.5 cm precision) for length, electric baby scales (0.01 kg precision) for infants' weight under 2 years old or electric body scales (0.1 kg precision) for weight of children over 2 years old. Weight was measured with minimum clothing (e.g. shorts or trousers) but no shoes and headcovering. Spaces at the community school or the community centre were used for the measurement.

The same two observers, the researcher and a field assistant, carried out all measurements with the same rules. Therefore, the intra-technical error of measurement (intra-TEM) and coefficient of reliability (*R*) were calculated by repeated measurements with 12 children (Ulijaszek and Kerr, 1999), before all the measurements of height, length and weight. Formula 1 below was used for intra-TEM calculation (Ulijaszek and Lourie, 1994).

$$TEM = \sqrt{\frac{\sum D}{2N}}$$

(Formula 1)

D is the difference between measurements and *N* is the number of individuals measured. In this study, TEM was 0.302 for weight, 0.238 for height and 1.237 for length. *R* was calculated using Formula 2.

$$R = 1 - \frac{TEM^2}{SD^2}$$

(Formula 2)

SD is standard deviation. $R>0.95$ is acceptable (range from 0 to 1). *R* was 0.999 for height, 0.999 for length, 0.992 for body scale weight and 0.999 for baby scale weight in this study. Therefore the technical measurement error did not disturb the accuracy of measurement in this study.

Generally, Nepali people in urban areas know their birthday taken from the Nepali calendar. The children's birthdays were ascertained through interviews, by at least twice with the mother/guardians using the Nepali calendar, and carefully calculated (e.g.

by asking siblings' ages and the gap between these and the child examined, as well as the mother's age at particular events, for instance, at marriage). The obtained Nepali birthday was converted to the Gregorian birth date using a special conversion calendar (trade edition). Using the Gregorian birthday, age, sex, HAZ and WAZ were calculated using EPI-NUT in EPI-INFO (version 6.03, 1996, CDC, USA).

3.3. Intestinal Permeability Test

3.3.1. Measures of Gut Function and Food Absorption

Ford et al. (1985) found that abnormal small intestinal morphology damaged by intestinal diseases was associated with increased permeability; they showed a strong correlation between crypt depth and permeability. The damage of mucosal function, particularly between cell walls in mucosa, therefore relates 'leaky' gut condition with hyper-permeability in the intestinal epithelium. Travis and Menzies (1992) said the intestinal epithelium demonstrates two differential properties: both 'transport' and 'barrier' functions. Thus, the integrity of small intestinal mucosa relates to the function of molecular food absorption as well as a local defense system against infectional pathogens.

The intestinal permeability assessment is measured directly using two different size and absorption sugar molecules - disaccharide (such as lactulose) and monosaccharide (such as mannitol) – in the ratio between the two sugars in urinary excretion. Lactulose is the most widely used disaccharide probe for intestinal permeability assessment. It is a synthetic disaccharide and too large a molecule to permeate into healthy mucosal cells (molecular weight: MW 340), therefore very little is absorbed in vivo (6-hour urinary excretion after 5g dose ingestion is under 0.25%; Behrens et al., 1987). Mannitol has a smaller molecular size (MW 180) and is transferred across the intestinal mucosa promptly (6-hour urinary excretion after 1g dose ingestion is up to 20%; Behrens et al., 1987). Both sugars are non-metabolized in vivo and are rapidly cleared by the kidneys at similar rates (Menzies, 1974). Both probes are absorbed by different routes across the intestinal wall; lactulose passes through only paracellular pathways (between the cells) and mannitol is absorbed transcellularly (across the cells). In the situation of hyper-permeability with leaky gut condition, absorption by the paracellular route is increased. The leaky gut condition shows a higher lactulose/mannitol ratio (L/M ratio) in urinary excretion than with a healthy gut function: the absorption of lactulose increases due to the leaky mucosal surfaces, whereas mannitol absorption decreases due to the reduction in absorption surface area in the mucosal abnormality. Therefore, the L/M ratio in urinary excretion is used as an index of the integrity of the small intestinal mucosa. These sugars are non-invasive to the body when taken orally, particularly suitable for children, and the intestinal permeability test supports biopsy (Lifschita and Shulman, 1990) and the dual-sugar intestinal permeability test can be assessed frequently on large numbers and recommended for clinical practice or even in field conditions (Cooper, 1984; Lunn et al., 1991b; Travis and Menzies, 1992; Bjarnason et al., 1995).

3.3.2. ***Procedure***

Children were given 400mg of lactulose (Duphalac; Duphar, Southampton, UK) and 100mg of mannitol (Sigma, Poole, Dorset, UK) dissolved in 2ml of water per kg of body weight. The amount of solution was a small quantity (e.g. only 40 ml of solution for child with 20kg of weight) and sweet enough for children to take readily. The test was scheduled 2 hours after breakfast and the children abstained 1 hour from eating foods. Breastfeeding, however, was not stopped, because the babies were too young for it.

Urine was collected in urine bags (U-bag Urine Specimen Collector Pediatric, Hollister Ltd., Berkshire, UK), or potties wrapped in clean plastic bags to avoid contamination (or older boys can simply urinate into a collecting bottle), depending on how children usually urinated (Figure 3-2). A few drops of bacteriostat (chlorhexidine digluconate, 20% aqueous solution) were added at the time of first collection of urine in a collection bottle. Total urine volume was recorded and two 1ml aliquots were taken after checking for faeces contamination. The samples were immediately frozen at –20℃ at the local hospital (TUTH) in Kathmandu until shipment to the UK. A cooler bag with ice packs was used for the air shipment and once back in the UK the samples were replaced in a freezer within 48 hours.

The samples were analysed by enzymatic assay (Lunn et al., 1989; Northrop et al., 1990; Blood et al., 1991; Lunn and Northrop-Clewes, 1992) using a centrifugal spectrophotometer Cobas-Bio (Roche, Welwyn Garden City, UK) at Northern Ireland Centre for Diet and Health, University of Ulster, Northern Ireland.

Figure 3-1. Urine collection for a girl and a boy

3.4. Blood Spots for C-Reactive Protein

3.4.1. Measures of Infection Status Using Immune Response in The Body

Serum C-reactive protein (CRP) is one of several acute-phase proteins. Acute-phase proteins react to systemic tissue damages mainly caused by microbal infection, such as bacteria or protozoa (McCarthy et al., 1978) as mediating protection against pathogens during the period before the development of an adaptive innate immune system (Lydyard et al., 2000). The CRP secretion in serum is very low in the healthy body, therefore the elevated CRP concentration in serum with inflammatory diseases can be used as a way to assess the current inflammatory status of the diseases. CRP, in particular, responds to infection immediately and dramatically; the serum concentration increases about 1000-fold as early as 4 hours following tissue injury damaged by microbial infection (Thompson et al., 1992) and it remains elevated for a period of 24-72 hours (Calvin et al., 1988). The concentration of CRP reflects the acute inflammation activity better than other acute-phase proteins, because of its rapid rising following local tissue damages, and its declining is faster than that of other plasma proteins (Laurell, 1985; Kushner, 1982).

CRP is useful clinical information for diagnosis and monitoring the inflammation status of patients (Dahler-Eriksen et al., 1997). Ballou and Kushner (1992) defined the clinical values of serum CRP concentration: under 10mg/l is normal or an insignificant elevation caused by minor stresses such as common cold, asthma or pregnancy; 10-100mg/l is a moderate elevation caused by moderate stresses such as mucosal infection, malignancy or myocardial infarction; over 100mg/l is a marked elevation caused by major stresses such as acute bacterial infection or major trauma.

3.4.2. Technique of Blood Spotting

Blood spotting was used for determining the level of acute-phase protein, CRP, in blood. The child's finger (or heel for young babies) was pricked with disposable lancet (HemoCue lancet, HemoCue Ltd., Angelholm, Sweden) and about four drops of blood collected on a filter paper which is commonly used for neonatal screening tests (Guthrie cards, Schleicher & Schuell, Keene, NH, USA). Hands or feet were soaked in warm water for a while before pricking to stimulate blood circulation. This method was non-invasive and caused minimal pain especially for young children. The spots were dried overnight in room temperature then frozen at $-20°C$ in the local hospital (TUTH). The samples were sent to Northwestern University in USA within 3 days (Four days posting did not affect the CRP concentration in blood spots; Cordon et al., 1991) then analysed using enzyme-linked immunoabsorbent assays (ELISA) methods (McDade et al., 2000).

The blood spot method for detecting CRP values is field-friendly and also reliable; serum/plasma and blood spot values of CRP show high agreement (Cordon et al., 1991; McDade et al., 2000). Blood spot uses whole-blood, not serum or plasma, therefore a blood spot value of 5mg/l, equivalent to a plasma value to 5.9mg/l, was used as a cut-off point for elevated CRP values indicating mild/high clinical/sub-clinical inflammation status (McDade et al., 2000).

3.5. Interviews - Reported Morbidity and Weaning Practices

Information on morbidity and weaning practices of children were collected by interview. To obtain this information on morbidity, open-ended questions by a local female assistant were put to mothers/guardians regarding whether or not the child was ill yesterday or in the preceding 7 days. If the child was ill, then further questions were asked regarding the nature of the illness according to common descriptive terms used by local rather than medical definitions, such as *pakhala* (diarrhoea), *khoki* (cough), *ruga* (cold or flu), *jwaro* (fever), *tawko dukhey* (headache), *pet dukhye* (stomach pain), *wakwaki* (nausea), *bomi* (vomiting), *juka* (worm infection) and *dadura* (measles) (Gartoulla, 1998).

Information on weaning practices were collected using questions regarding present feeding status - whether the child was breastfed or given solid food or both together. The timing of first supplementation of solid foods, such as *pito* (gruel with lentil beans and rice) or *bhat* (cooked rice), and cessation of breastfeeding were collected. Almedom (1991) defined the process of weaning as two different stages: (1) the introduction of supplementary foods on a regular basis, and (2) the completion of weaning when breastfeeding is ceased. This study follows Almedom's definition of weaning.

3.6. Stool Sampling for Parasite Infection

Stool samples were collected for parasite infection by the mothers/guardians, using a standard procedure which is familiar to local people in urban areas. The mothers inserted a stick at several places in the excreted faeces, and the faeces collected were put into a specimen case (film case) and sealed. Samples were brought daily to the Clinical Microbiology Department (TUTH), and examined using standard microscopy techniques.

3.7. Sample Sizes Calculation

In the preparation of sample sizes, anthropometric status and intestinal permeability tests were calculated according to a formula given by Mascie-Taylor (1994:58). The calculation of sample size depends upon the chosen level of significance, the power of the test, the variance and expected difference (*diff*) between group means in a given population. For a two-tailed test, a level of significance set at 0.95, and a power of the test set at 80%, the Formula 3 becomes:

$$\frac{2 * S^2 (1.96 + 0.842)^2}{diff^2}$$

(Formula 3)

To determine poor anthropometric status, a z-score of –2 or below in HAZ or WAZ is used. A difference of 0.33 in the z-score of two populations determines a genuine difference in averages (Mascie-Taylor, personal communication). The expected variance (squared standard deviation) for HAZ was taken from a study by Panter-Brick (1997) in Nepal. For 1-3 year olds, if S = 1.23, the sample size is calculated as 218. For infants of 0-11 months, if S = 0.77 the sample size is calculated as 85.

For intestinal permeability tests, a difference of L/M ratio was taken as 0.09 and the standard deviation was 0.13 from Rousham et al. (1998). Therefore, the calculated sample size was 33.

Sample size for CRP analysis, however, could not be calculated because there is no information in the literature of standard deviation, as 95% CI is often shown in previous studies due to the nature of distribution.

3.8. Statistical Analyses

210 children were measured with regard to their weight and height/length in the total sample of 0-60 months old. Five children were uncooperative at the measurement of weight and height and would not adopt the correct position, so they were measured by

length instead of height and weighed with the mother and then the mother's weight excluded. Two children had no birth dates, so the day in the middle of the birth month (15th) was used for a proxy date of birth. Under –3.50 z-scores were recognised as outliners. Therefore, HAZ data on 199 children (11 outliners) and WAZ data on 210 children were used for analyses of anthropometric status.

173 children's blood spots for CRP and faeces for detecting parasitic infections and 172 morbidity reports and items of weaning information were collected. The other 38 children were either moved out from the communities or refused testing after the anthropometric measurement. 169 urine samples from children were obtained for the intestinal permeability test.

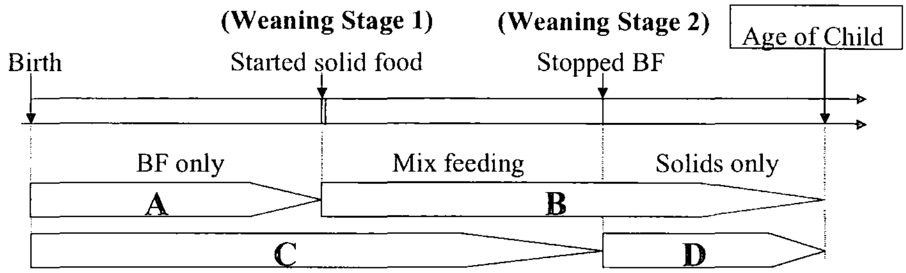
Intestinal permeability was expressed as the ratio of the sugars, lactulose and mannitol (L/M ratio). Values for lactulose excretion and mannitol excretion were accepted if they were within the range of expected recovery rates [(urine excretion of sugar / actual intake of sugar) x 100]. The percentage recovery rate for lactulose was 0.02-15.00 and for mannitol 0.5-15.00. Therefore, 11 outliers of lactulose and mannitol were outside of the recommended ranges of the two sugars. Therefore, the L/M ratio for 158 children were used for analysis.

Lactose and creatinine (a proxy for urinary function) levels were also examined. Values of lactose/lactulose ratios above 0.4 were taken to indicate significant lactose maldigestion (after Northrop-Clewes et al., 1997). Values of lactose for 168 children and lactose/lactulose ratio of 157 children (values of lactose/lactulose ratio for 158 children were obtained but 1 outlier of lactose was excluded) were used for analysis.

Intestinal permeability ratios and lactose values were converted to natural logarithms to normalise the data distribution (after the Kolmolgorov-Smirnoff statistic indicated significant skewness). The geometric mean for the raw L/M ratio (i.e. the anti-log of mean log L/M ratio) was used for the purposes of comparison with previous studies. As a measure of variance, a value termed the geometric standard error (SEM), defined as [antilog (mean + standard error of logged values) – geometric mean], was also calculated. The published norm for healthy UK children (Lunn et al., 1991a) - geometric mean 0.12 (corresponding to log L/M ratio of –2.12) - was taken to demarcate between poor and good intestinal permeability; a high L/M ratio (i.e. over log L/M ratio –2.12) indicates poor gut function.

CRP data were categorised to indicate high (dummy variable: 1) or low (0) inflammatory status, using a cut-off point of 5mg/l in blood spot value (see 2.4.2. Technique of Blood Spotting, p. 29).

Weaning practices were coded as four variables: A) duration of exclusive breastfeeding, B) duration of supplementation, C) time until stopped breastfeeding and D) time on solids only (Figure 3-2). Thus the timing of weaning is given by variables A and B (stage 1: introduction of solid foods) and variables C and D (stage 2: cessation of breastfeeding).



(BF: breastfeeding)

(**A**) Duration of exclusive BF
(**B**) Duration of supplementation
(**C**) Time until stopped BF
(**D**) Time on solids only

Figure 3-2. Four different variables characterised feeding practices

Parasite infestation and its type were recorded and coded as children with no infection (dummy variable: 0), helminth (1) and Giardia (2) infection. Morbidity data were coded as children who were ill (1) versus not ill (0), and also had diarrhoea (1) versus not (0), in the previous day and the preceding 7 days.

LSD (least significant difference) post hoc tests were used to evaluate differences between sub-groups of children. Stepwise multiple and logistic regression analyses were used to explore relationships between anthropometric status, intestinal permeability, acute-phase protein (CRP), feeding practices, parasite infection, morbidity and children's characteristics, such as age, sex, caste/ethnicity (Tibeto-Burmese vs. Indo-Aryan - high and low caste) and squatter location (the two sample areas).

Data analyses were undertaken using the Statistical Package for the Social Sciences (SPSS, version 10.0.7), and $p<0.05$ was the accepted level of significance.

3.9. Ethical Condition

Ethical permission for the study was granted by the Durham University Ethics Advisory Committee in July 1999 and by Tribhuvan University in Nepal in September 1999. One must note that the methods of blood spot and intestinal permeability tests with urine have been reported as successful non-invasive techniques in previous studies (e.g. in Bangladesh for 2-5 years-old; Rousham et al., 1998). In the current fieldwork, too, these methods proved successful. A health education worker from Lumanti introduced this investigation to the subjects as an opportunity to carry out health checks for worm infection, anaemia and basic urine tests, explaining the process and the reasons for the tests in detail to parents and children before gaining their permission. Consent was verbal, because many participants were illiterate. Families were free to refuse participation at any time. The results of hemoglobin level using a portable haemoglobin checker, parasite infection and basic urinary tests analysed in the local hospital (TUTH) were shown on paper and explained to mothers/guardians. Also, following the research results, a health education programme was conducted by a health worker in Lumanti. De-worming medication and iron supplements (donated by Simca Ltd., Kathmandu) were given to mothers and children in whom problems were found. In the case of severe problems being discovered the mother would be advised to visit hospital.

3.10. Funding

The Department of Anthropology at the University of Durham supported expenses for research equipment. Rotary International supplemented a fund for personal subsistence expenses in Nepal. The Sir Richard Stapley Education Trust supported the research and provided some subsistence funds during fieldwork. The Infant and Child Research Group (ICRG) funded the laboratory analysis.

3.11. Collaboration

Dr. Catherine Panter-Brick supervised and set the base design for the research using her previous experience of fieldwork in Nepal on child growth and biochemical markers of infection in both urban and village settings. Dr. Shyam Thapa at Family Health International in Kathmandu offered advice and helped to start this research. Dr. David Osrin and Dr. Dharma Manandhar at the Mother and Infant Research Activities (MIRA) project in Kathmandu gave me much advice about the research plan and equipment. Dr. Christine Northrop-Clewes and Dr. Anne Susan Mburu at Northern Island Centre for Diet and Health in the University of Ulster, Northern Island, analysed urinary intestinal permeability and supported training in the techniques used. Dr. Thomas McDade at the Northwestern University in Chicago in the United States analysed blood spots for CRP. Dr. Renu Manandhar and Dr. Nhuchhe R. Tuladhar at the Department of Clinical Microbiology in Tribhuvan University analysed parasite infection from faeces samples. Dr. Robert Drewett in the Department of Psychology in Durham University kindly gave statistical advice.

CHAPTER 4

RESULTS

4.1. Overview

A number of questions are raised in this study: Can biochemical markers explain poor anthropometric status in Nepal? Are anthropometric status variables or biochemical markers associated with morbidity reports, parasite infection or weaning practices? This chapter presents data on anthropometric status and biochemical markers, such as intestinal permeability (food absorption) and acute-phase protein (inflammation status). Weaning practices are shown to be variable for child anthropometric status in poor urban settings in Nepal.

4.2. Sample Characteristics

There were similar numbers of males and females and of children per location: 98 boys and 112 girls; 108 in Pathibhara and 102 in Balaju. There were also similar numbers of children in each age group (Appendix 2: Table 1). Mean age of children was 45 months old (SD 10.96) (Table 4-1) and over one half of the children were of Tibeto-Burmese origin (60%), others being of Indo-Aryan origin, either high caste (24%) or low caste (16%) children (Appendix 2: Table 2). Muslims and Indians were excluded for this study, as they were only 1%.

4.3. Anthropometric Status

The squatter children were mildly stunted and underweight (mean HAZ −1.45, WAZ −1.62; Table 4-1). Children also showed a high proportion of malnutrition: 37% and

33% for HAZ and WAZ below −2 z-scores respectively.

In Figure 4-1, HAZ and WAZ were plotted with children's age in the total 0-60 months old group. It is clear that their growth faltering starts early on in life. Means of HAZ and WAZ in each age group are shown in Table 4-1. The faltering in the early stage of life was much faster than the later stage. Anthropometric status failed below −1.50 z-scores after 12 months of age in both HAZ and WAZ: children showed better anthropometric status in the early stage of life (0-6 months old; HAZ −0.22, WAZ −0.17) and the two mean z-scores were significantly higher than the other age groups ($p<0.0001$). Additionally, WAZ failed faster than HAZ: mean z-score of WAZ between 0-6 and 6-12 months old showed significant difference ($p=0.007$), whereas mean HAZ between the two age groups did not show significant difference.

Table 4-1. Age and growth status of Nepali squatter children

Variable	Number	Mean	Median	SD	Range
Age (month)	210	45.19	46.90	10.96	0.30 - 59.93
HAZ	199†	−1.45	−1.61	1.16	−3.43 - 2.83
WAZ	210	−1.62	−1.66	0.83	−3.15 - 1.06

Age Groups (month)	Mean HAZ (SD)	Mean WAZ (SD)
0-5.99	−0.22 (0.83)	−0.17 (1.20)
6-11.99	−0.67 (1.22)	−1.00** (1.09)
12-23.99	−1.51* (1.08)	−1.51* (0.86)
24-35.99	−1.57* (0.85)	−1.67* (0.81)
36-47.99	−1.88* (0.91)	−1.61* (0.71)
48-60.00	−1.82* (1.28)	−1.49* (0.85)

NB: 0-11.99 month: mean HAZ −0.50 (1.10), mean WAZ −0.67 (1.19)

* $p<0.0001$; compared to 0-12 months old children (LSD post hoc tests)

** $p=0.007$; compared to 0-6 months old children (LSD post hoc tests)

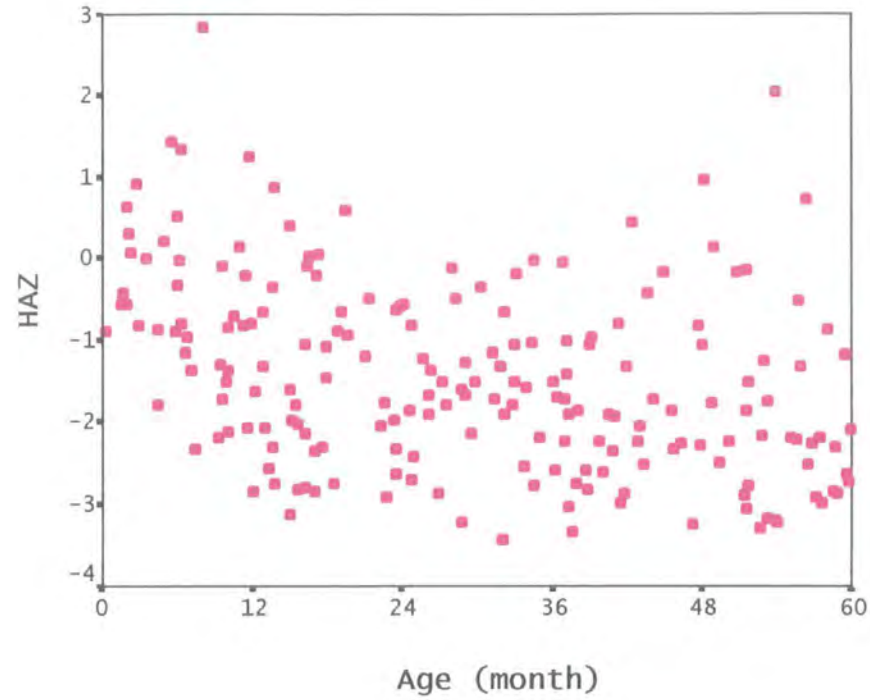
† 11 outliers were excluded.

Age correction is incorporated in the z-scores, HAZ and WAZ, though age alone made a strong impact on HAZ (R^2=16%, p<0.0001), while both age and ethnicity had an impact on WAZ (R^2=14%, p<0.0001 and p=0.006 respectively). Tibeto-Burmese children achieved better anthropometric status than the low caste children of Indo-Aryan origin (ANOVA p=0.032; LSD post hoc tests between Tibeto-Burmese and Indo-Aryan low caste p=0.009 for WAZ), especially after 18 months old: ethnicity, not age, gave an impact on anthropometric status in the over 18 months old group (R^2=6%, p=0.003) (Table 4-2). Sex and location made no detectable differences in the findings.

Table 4-2. Different profile of anthropometric status (WAZ) in children below and over 18 months old

Independent variables	R^2	B	SE	Sig.
1) Children below 18 months				
Age	0.269	-0.117	0.024	<0.0001
2) Children over 18 months				
Ethnicity (Tibeto-Burmese)	0.061	0.441	0.147	0.003

Dependent variable: WAZ; Independent variables include age (month), sex, location (two sites), ethnicity, and age-ethnicity interaction



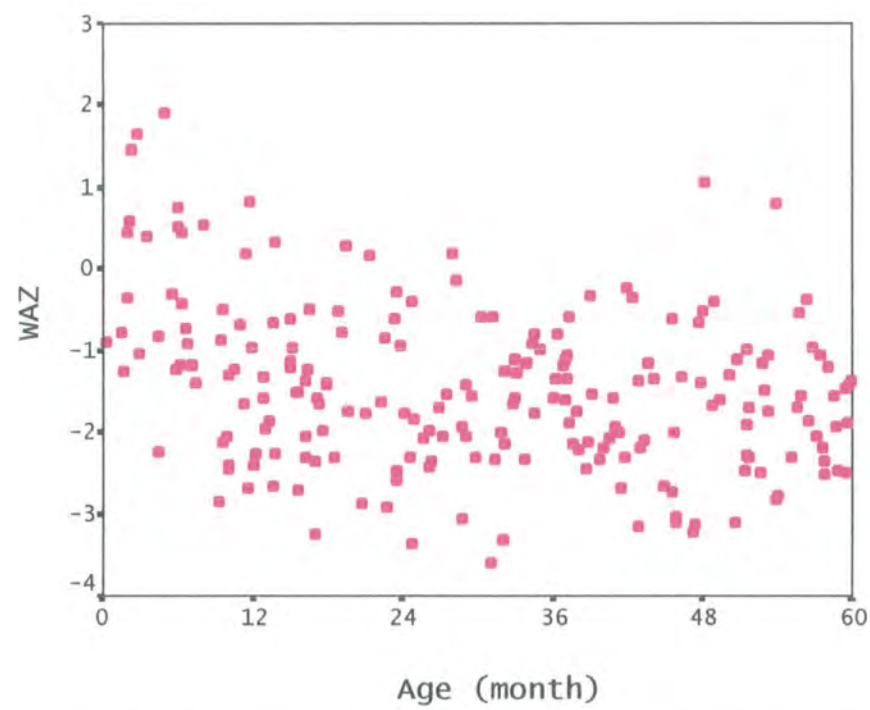


Figure 4-1. HAZ and WAZ distribution in all children (0-60 months old)

4.4. Intestinal Permeability

4.4.1. Levels of Intestinal Permeability in Nepali Squatter Children

The actual raw data for L/M ratio show a wide range of values (Figure 4-2), all within the range of acceptable recovery rates (Table 3 in Appendix 2 shows raw and log values of L/M ratios).

Child age, sex, ethnicity and location had no impact on the values of L/M ratio.

Most children (92%) had poor intestinal permeability compared to the UK norm of geometric mean 0.12 (Lunn et al., 1991a). Nepali children had similar intestinal permeability levels to a sample of rural Bangladeshi children (Northrop-Clewes et al., 2001; L/M ratio in geometric mean 0.26 for Nepal and 0.24 for Bangladesh, see Table 5-1, p.58).

4.4.2. Lactose Maldigestion in Nepali Squatter Children

One half of children studied (47%) showed low lactase activity (lactose/lactulose ratios over 0.4). The values of lactose and lactose/lactulose ratio are shown in Table 4 in Appendix 2. Values of lactose values and lactose/lactulose ratios decreased with age (R^2=28%, $p<0.0001$); sex, ethnicity and location had no impact.

Lactose values and lactose/lactulose ratios showed no associations with L/M ratios.

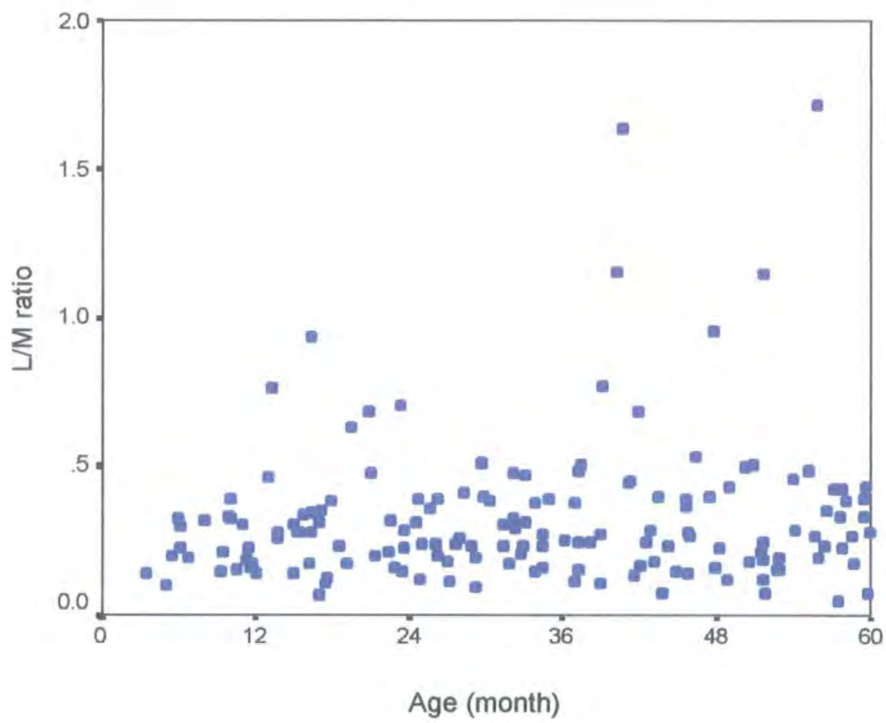


Figure 4-2. Values of intestinal permeability in Nepali children

(r= 0.130, p=NS, n=158)

4.5. C-Reactive Protein (CRP)

The distribution of CRP data was skewed (Kolmolgorov-Smirnoff test, $p<0.0001$; mean 1.85, median 0.00, SD 4.10, range 0-23.2); therefore the data were categorised according to levels of inflammation (Table 4-3). 16% of children had over 5 mg/l (blood spot value) indicating mild/high clinical/sub-clinical inflammation status.

Table 4-3. Levels of CRP in the Nepali squatter children

CRP (mg/l)*	0-4.9	5-9.9	Over 10
Number (%)	145 (83.8)	15 (8.7)	13 (7.5)

*whole-blood spot value, not serum equivalent

The levels of CRP were associated with age and location (logistic regression): thus younger children and children in Balaju showed higher CRP values (R^2=6%, p=0.014 and p=0.021 respectively). Sex had no impact on the level of CRP.

4.6. Morbidity

4.6.1. Morbidity in Nepali Squatter Children

Of the 172 children, 24% were reported ill on the day of interview and 41% children had been ill in the preceding 7 days (Table 4-4). Diarrhoea and respiratory diseases were most common among the children, followed by fever, infections and vomiting.

Table 4-4. Morbidity reports on the day of the interview and the preceding 7 days

Morbidity reports	The day of the interview no. of children (%)	The preceding 7 days no. of children (%)
Respiratory diseases	19 (34.5)	25 (28.7)
Diarrhoea	17 (30.9)	23 (26.4)
Fever	10 (18.1)	17 (19.5)
Infections	2 (3.6)	6 (6.9)
Vomiting	1 (1.8)	6 (6.9)
Others	6 (10.9)	10 (11.5)
Total		
Ill	**42 (24.4)**	**71 (41.3)**
Not-ill	**130 (75.6)**	**101 (58.7)**

NB: respiratory diseases: *khoki* (cough), *ruga* (cold or flu), pneumonia, sore throat; diarrhoea: *pakhala*; fever: *jwaro*; infections: ear, eye, skin, tongue and worm (*juka*) infection; vomiting: *bomi*; others: *tawko dukhey* (headache), *pet dukhye* (stomach pain), *wakwaki* (nausea), less appetite

Illness in the preceding 7 days was negatively associated with child age (R^2=3%, p=0.033), and diarrhoea in the preceding 7 days was associated with both age and ethnicity (R^2=7%, p=0.010): thus young children were more likely to be ill and Tibeto-Burmese children more likely to have diarrhoea. Neither sex nor location had an impact on morbidity.

4.6.2. Morbidity and Lactose Maldigestion

Illness in the day of interview had no detectable impact on L/M ratios, but was associated with higher lactose values and also higher lactose/lactulose ratios (R^2=31% and 36% respectively, p<0.0001, controlling for infant age). Specially, having diarrhoea was associated with higher lactose and lactose/lactulose values (p<0.001).

4.7. Parasitic Infection

Parasite infection was detected in 54% of the 173 children whose stools were examined. *Ascaris lumbricoides* (roundworm) was most common, followed by *Trichuris trichiuria* (whipworm), *Giardia lambia* (Giardia), hookworm, and *H. nana* (tapeworm) (Table 4-5) (see Appendix 1).

Table 4-5. Type of parasite infection found in squatter children

Type of parasite	Number of children (%)
Ascaris lumbricoides (roundworm)	55 (58.5)
Trichuris trichiuria (whipworm)	35 (37.2)
Giardia lambia (Giardia)	13 (13.8)
Hookworm	8 (8.5)
H. nana (tapeworm)	7 (7.4)
Entamoeba histolytica	2 (2.1)
Children infected by	
any parasites	94* (54.3)
no parasite	79 (45.7)
TOTAL	173 (100.0)

*24 children were infected by more than two parasites.

Child age and location were significant predictors of parasite infection (logistic regression, R^2=20%, p<0.0001 and p=0.002 respectively): thus older children and children in the community most easily flooded by river water showed higher rates of infection.

4.7.1. Giardia Infection and Intestinal Permeability

Children with Giardia showed significantly worse L/M ratios (geometric mean 0.43 for Giardia-infected versus 0.25 for non-infected children), although a sample of only 8 children[1] were infected with this protozoan was used (post-hoc tests, p=0.014 for Giardia versus non-infected children; p=0.03 for Giardia versus helminth infections; non significant for non-infected versus helminth infections; Figure 4-3).

[1] Only 8 children of the 13 children infected Giardia had data on L/M ratio.

4.7.2. Intestinal Permeability with Morbidity and Parasitic Infection

Neither parasite infection nor morbidity made a detectable impact on intestinal permeability where examined as a continuous variable on L/M ratio.

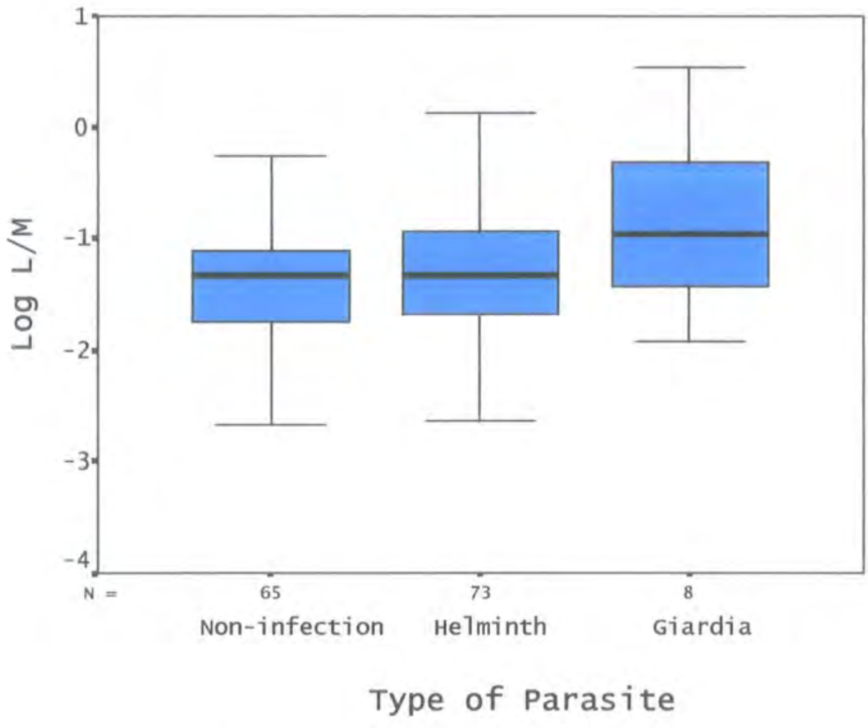


Figure 4-3. Impact of parasitic infection on intestinal permeability

4.8. Weaning Practices

Only two out of 172 children had been not-breastfed from birth. Children started supplementary weaning foods at 6 months (variable A: mean 5.9 (SD 3.9) months) and stopped breastfeeding when 2 years old (variable C: mean 23.0 (SD 11.6) months). At the time of the study, the duration of supplementation was 27 months (variable B: N=170, mean 26.6, SD 16.6), and the average time on solids was 19 months (variable D: N=83, mean 19.1, SD 12.4).

Stage 1 of weaning (the onset of food supplementation) was associated positively with only child age (R^2=95%, $p<0.0001$ for variable B). Stage 2 (the cessation of breastfeeding, variables C and D) was associated with age and location (i.e. children in Pathibhara stopped breastfeeding later) (R^2=41%, $p<0.0001$ and $p=0.031$ respectively for variable C; R^2=33%, $p<0.0001$ and $p=0.018$ respectively for variable D). Sex, caste and ethnicity of the child had no detectable association with weaning practices.

4.8.1. Intestinal Permeability with Weaning Practices

Two groups of children, breastfeeding and non-breastfeeding, were considered separately. Among the former, weaning practices showed no association with intestinal permeability (Table 4-6). Among the latter, variable C accounted for 10% of the variance in the data ($p=0.031$). Age, parasite infection and morbidity made no additional impacts on intestinal permeability.

Table 4-6. Two regression analyses for intestinal permeability (Log L/M ratio)

Independent variables	R^2	B	SE	Sig.
Stage 1. Breastfeeding group (N= 44)				
All variables	-	-	-	NS
Stage 2. Non-breastfeeding group (N=48)				
Weaning practice (variable C)	0.095	0.017	0.008	0.031

Independent variables include age (month), weaning practices (month), parasitic infection, and morbidity

Figure 4-4 illustrates the positive relationship found for variable C: children who had experienced a longer period of breastfeeding showed worse gut function (i.e. higher L/M ratios) than children who had breastfed for less time. Thus for this sample, stage 2 of weaning, but not stage 1, had a significant impact on levels of intestinal permeability. These results were not confounded by age.

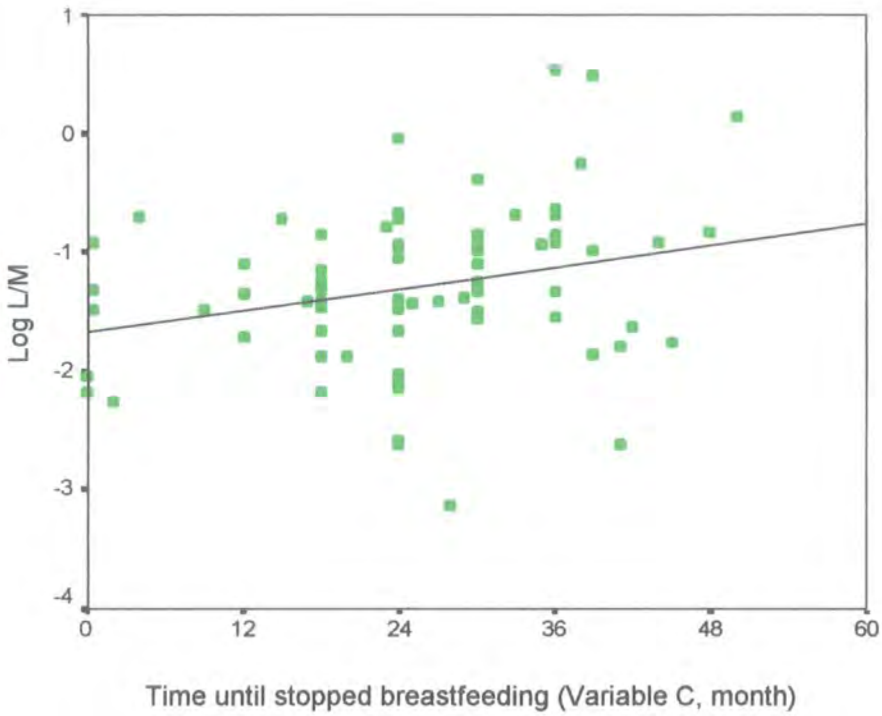


Figure 4-4. The negative relationship between L/M ratio and the time until stopped breastfeeding (variable C)

(r= 0.271, p=0.019, N=75)

4.8.2. Lactose Maldigestion and Weaning Practices

Breastfed children had significantly higher lactose values and lactose/lactulose ratios than their non-breastfed counterparts (lactose 17.25 and 4.45mg/dl; lactose/lactulose ratios 2.76 and 0.31, respectively) ($p<0.0001$, corrected for infant age).

In the breast-fed group of children, lactose values were negatively associated with stage 1 of weaning (duration of supplementation, variable B) along with morbidity on the day of interview ($R^2=30\%$, $p=0.001$ and $p=0.034$ respectively). Lactose/lactulose ratios were also associated with the same variable ($R^2=42\%$, $p<0.0001$ and $p=0.005$ respectively).

4.9. Morbidity Reports and CRP Profile

CRP values were associated with morbidity reports on the day of the interview, along with ethnicity and age ($R^2=10\%$, $p=0.001$, Table 4-7). However, the morbidity reports in the preceding 7 days were not associated with the levels of CRP.

Table 4-7. Regression analysis for inflammation status (CRP)

Independent variables	R^2	B	SE	Sig.
	0.100			
Morbidity (day of interview)		0.171	0.064	0.008
Ethnicity		-0.167	0.064	0.009
Age		-0.003	0.002	0.042

Dependent variables: CRP (low/high); Independent variables include age (month), ethnicity, location (two areas) and morbidity (the day of the interview/the preceding 7 days), N=167

4.10. Anthropometric Status and Biochemical Markers with Conditions of Weaning Practices

With respect to anthropometric status, in the group of breastfeeding children, the only variable of importance was ethnicity and its interaction with age (HAZ and WAZ, Table 4-8). Among non-breastfeeding children, however, weaning stage 2 (variable C) had a strong negative impact on HAZ (p=0.028); ethnicity and age had an impact on WAZ (p=0.007 and p=0.049, respectively) (Table 4-8). Thus, a longer period of breastfeeding was associated with poorer HAZ; intestinal permeability, parasite infection and reported morbidity had no detectable impact on anthropometric status.

Table 4-8. Two regression analyses for anthropometric status (HAZ and WAZ)

1) Breastfeeding group (N=42 for HAZ, 43 for WAZ)

Independent variables	Dependent variables	R^2	B	SE	Sig.
	HAZ	0.222			
Ethnicity			1.804	0.535	0.002
Ethnicity and age interaction			0.044	0.018	0.019
	WAZ				
All variables		-	-	-	NS

2) Non-breastfeeding group (N=42 for HAZ, 48 for WAZ)

Independent variables	Dependent variables	R^2	B	SE	Sig.
	HAZ	0.114			
Weaning Variable C			-0.026	0.012	0.027
	WAZ	0.125			
Ethnicity			0.623	0.241	0.013

Independent variables include age (month), ethnicity, intestinal permeability (Log L/M), lactose (Log), CRP (low/high), weaning practices (month), parasitic infection (non-infection, helminth and Giardia infections), and morbidity

4.11. Summary

This study showed that Nepali squatter children between the ages 0-60 months old were:

(1) Mildly stunted and underweight, failing below −1.5 z-scores after 12 months of age. WAZ values fall faster than HAZ. Tibeto-Burmese children

showed better anthropometric status in WAZ after 18 months old.

(2) Most (92%) children showed poor intestinal permeability levels and Nepali children showed similar levels to rural Bangladeshi children. One half of the children (47%) showed lactose maldigestion. Lactose and lactose/lactulose ratios decreased with age. Lactose maldigestion and levels of intestinal permeability had no association.

(3) 16% of children had milder/high clinical/sub-clinical inflammation.

(4) High prevalence of morbidity was found: one quarter of the children (24%) were reported ill during the day of interview and nearly one half of the children (41%) had been ill during the previous 7 days. Respiratory diseases and diarrhoea were most common. Younger children were more likely to be ill than the older one and Tibeto-Burmese children were more likely to have diarrhoea. Illness, especially diarrhoea, was associated with lactose maldigestion.

(5) One half of the children (54%) were infected by parasites. *Ascaris lumbricoids* and *Trichuris trichiuria* infection were most common. The children who were older and who lived in the community most easily flooded by the river showed higher parasitic infection. Children infected with Giardia showed significantly poorer intestinal permeability than others.

(6) Neither parasite infection nor morbidity had any impact on intestinal permeability.

(7) Children started being fed supplementary weaning foods at 6 months and stopped breastfeeding when 2 years old. In the non-breastfeeding group, children who had experienced a longer period of breastfeeding showed worse gut function (i.e. a higher L/M ratio). Breastfed children had higher

lactose and lactose/lactulose ratios. In the breastfed children, both lactose values and lactose/lactulose ratio were negatively associated with the duration of supplementation, along with morbidity on the day of interview.

(8) CRP values were associated with morbidity on the day of interview, along with age and ethnicity, but not with morbidity in the preceding 7 days.

(9) Among the non-breastfeeding group, weaning practice (variable C: the time until stopped breastfeeding) was associated with poor HAZ. Intestinal permeability, acute-phase protein, parasitic infection and reported morbidity had no detectable impact on anthropometric status.

CHAPTER 5

DISCUSSION

5.1. Overview

This chapter summarizes basic findings and compares these findings with the literature. The study examines the relation between poor child anthropometric status in urban Nepal and status of food absorption, inflammation and parasite infection as well as weaning practices. It tries to throw light on the mechanism of poor anthropometric status and to build a picture of the environmental factors in the lives of Nepali children.

5.2. Anthropometric Status

The children in this study were mildly stunted and underweight. Their anthropometric status failed rapidly before 12 months of age. This is a similar trend to that reported by Moffat who measured 0-60 month-old children in urban poor in Kathmandu (1998); age of 6-12 and 12-24 months: HAZ −1.48 and −1.97, WAZ −1.34 and −1.72 respectively (Moffat, 2001:311). There were no detectable sex differences (e.g. in Nepal: Martorell et al., 1983; Panter-Brick 1997).

It was found that Tibeto-Burmese children fared better after 18 months of age. This difference between ethnic groups has been reported in other studies. Thus Strickland and Tuffrey (1997) also found better anthropometric status in Tibeto-Burmese children at an early stage of their life compared to those of Indo-Aryan origin in Nepal. The authors described poor weight increases, between the ages of 1 to 3 years, with a mean value of WAZ −2 in Mongoloid children and −2.5 in non-Mongoloid children. In contrast to weight, patterns of growth in height (HAZ) were similar in both Mongoloid and non-Mongoloid children.

5.3. Intestinal Permeability

5.3.1. Levels of intestinal permeability in Nepali squatter children

As expected, most of the Nepali children showed poorer mucosal function (food absorption) than UK children (Lunn et al., 1991a). Values of intestinal permeability in different countries and anthropometric status from the literature are shown in Table 5-1. The geometric mean of 0.26 for the Nepali children was very similar to that obtained for rural Bangladeshi children who had been differentiated into subgroups of 'healthy' and 'ill with diarrhoea' individuals, with geometric mean values of 0.23 and 0.32 respectively (Rousham et al., 1998) (Table 5-1).

Table 5-1. Values of intestinal permeability from the literature

Location and Sample	Growth and Illness Status	Geometric mean L/M ratio	References
UK 3-15 months old (N=60)	Healthy	**0.12** (SEM 0.02)*	Lunn et al. (1991a)
Bangladesh, rural 2-5 year-olds (N=100)	HAZ -3.00 (±SD1.32) WAZ -2.70 (±SD0.74)	**0.24**	Northrop-Clewes et al. (2001)
2-5 yr old (N=352)	Healthy	**0.23** (95% CI 0.22-0.24)	Rousham et al. (1998)
(N=25)	Ill with diarrhoea	**0.32** (95% CI 0.24-0.43)	
Nepal, urban squatter 0-4 year-olds (N=167)	HAZ -1.45 (SD 1.16) WAZ -1.62 (SD 0.83)	**0.26** (SEM 0.21)*	R. Goto et al. (this study)
The Gambia 3-15 month-olds (N = 119, in 922 tests)	Healthy	**0.38** (SEM 0.01)*	Lunn et al. (1991a)
0-18 month-olds (N=60)	WAZ of 50th centile NCHS >80%	**0.42** (± 2SD 0.2-1.4)	Behrens et al. (1987)
(N=27)	<60%	**1.3** (±2SD 0.2-13)	
(N=47)	Ill with diarrhoea	**1.3** (±2SD 0.2-10.4)	
Guatemala 0-11 months (N=200)	No diarrhoea	Median value 0.05	Goto et al. (1999)
(N=10)	With diarrhoea	Median value 0.09	

*SEM = geometric standard error which is defined as: [antilog (mean + standard error of logged values) – geometric mean]

5.3.2. Intestinal Permeability and Diarrhoea

Unlike this study, Weaver et al. (1985) found that increased L/M ratio was a sign of mucosal damage by micro-organism infection and several studies support a strong relationship between a current episode of diarrhoea and poor gut function. In the Gambia, children who suffered from diarrhoea more than 14 days showed significantly poorer intestinal permeability than children with diarrhoea for less than 14 days (geometric mean 2.85 and 1.0 respectively, $p<0.001$; Behrens et al., 1987). However, no significant differences were found between children who had diarrhoea and those reported healthy in this study (geometric mean 0.31 and 0.31 respectively). Other studies, however, have simply excluded children affected with acute diarrhea because of technical problems with urine collection (yet still found that recent history of diarrhea was associated with altered gut function; Goto et al., 1999). Other morbidity factors, such as fever or respiratory infection, were associated with variation in the levels of intestinal permeability (Rousham et al., 1998).

5.3.3. Intestinal Permeability and Giardia Infection

In the present study, more than one half of the children were infected by parasites and *Ascaris lumbricoids* and *Trichuris trichiuria* were most common; high prevalence of parasitic infection has been reported not only in rural and but also in urban Nepal, attributed strongly to people's lifestyle in different sections of the population; lifestyle factors would include eating habits, wearing of foot wear and sanitation conditions, particularly the use of unclean water, the efficiency of latrines and inadequate faeces disposal, and the practice of using human faeces as fertiliser. Suguri et al. (1985) found a high prevalence of parasite infection (87%) among Nepali people, compared with a control group of Japanese in Kathmandu (35%). Rai et al. (1994) reported parasitic infection in urban area using hospital records from 1985 to 1992. The annual prevalence of parasite infection among patients was 19% in 1992 (decreasing from 37% in 1985). Infection by Ascaris was the most common, followed by hookworm and Trichuris infections. Sherchand et al. (1996) found that 28% of children and 39% of adults had parasitic infection, but no symptom of abdominal discomfort (i.e. sub-clinical conditions of parasitic infection), among hospital patients in Kathmandu area.

This study showed that neither reported morbidity nor helminth infestations were convincingly associated with altered intestinal permeability. However, the presence of Giardia was significantly associated with poor gut function compared to absence of parasitic infection ($p=0.014$). There were only a few identified cases of hookworm ($N=8$) and Giardia ($N=8$), which are the parasites that cause the greatest damage to the intestinal wall (Solomons, 1982; Farthing, 1984, see appendix 1). Indeed, one recent study in Bangladesh reported a significant increase in the prevalence of Giardia in a group of children treated with antihelmintics, which was associated with higher values of L/M ratio and a short-term decrease in weight (Northrop-Clewes et al., 2001).

5.3.4. Lactose Maldigestion

A measure of lactose absorption, reflecting lactase activity, is also a useful indicator of maldigestion (Northrop-Clewes et al., 1997). For children, the most important source of lactose is breast-milk and cow's milk, and the sugar constitutes an important source of energy for growth. In this study, one half of the children showed lactose maldigestion, symptomatic of low lactase activity. Interestingly, breastfed children had significantly higher lactose values (and lactose/lactulose ratios) than those who were no longer breastfed ($p<0.0001$), but had similar intestinal permeability values (geometric mean 0.26 in breastfed children and 0.27 in non-breast feeding children). This may reflect higher lactose intakes in those children still breastfeeding, which fall after stage 2 of weaning. Lactose values were also positively related to morbidity reported for the day of interview, and particularly diarrhoea. Such observations could either reflect the fact that ill children breastfed more than usual, or that there is gut damage and poorer lactose absorption concurrent with diarrhoea.

5.3.5. *Timing of Weaning and Intestinal Permeability*

Children started eating supplementary weaning foods at 6 months and stopped breastfeeding at 2 years in this study. Moffat (2001) studied breastfeeding status among the urban poor in Kathmandu and found that all infants were introduced to supplementary foods by 7 months old and ceased breastfeeding by 3 years of age. More than one half of women stopped breastfeeding because of a near pregnancy. Regarding the behavior of infant feeding practices, there is a great variability in different ecological and cultural settings (Jelliffe and Jelliffe, 1978; Prentice and Paul, 1990) and the variability of feeding behavior within community is often observed to be wider than that between communities (Panter-Brick, 1992). Feeding practices in different settings are often influenced by several physical and social factors, such as availability of adequate supplemental foods, opportunities for breastfeeding, constraints of maternal work, social or family support, exposure to western medicine and formula feeding, beliefs about breastfeeding and supplementation, or understanding of child development (Jelliffe and Jelliffe, 1978; McDade and Worthman, 1998). In Moffat's study of feeding practices in Nepali urban poor, there were no detectable ethnic differences (Tibeto-Burmese versus Indo-Aryan origin) in the proportion of infants under 6 months of age that were exclusively breastfeeding (Moffat, 2001). The most common response of mothers about the timing of cessation of breastfeeding was 'it is not necessary to give food if milk is enough; if milk is not enough, we must feed (Moffat, 2001:328)'. Moffat concluded that those Nepali mothers seem to react to what they see to be the needs of their babies on a case-by-case basis.

The timing of weaning showed a significant impact on intestinal permeability. Interestingly, such impact was detected from the cessation of breastfeeding (stage 2) rather than from the introduction of supplementary foods (stage 1). To my knowledge, only one other study conducted in Guatemala (Goto et al., 1999) has specifically examined the associations between feeding practices and intestinal permeability. That study found that L/M ratios were correlated with the age at termination of breastfeeding (p=0.036), but were unrelated to the period of non-breastfeeding. The authors argued that 'it was the early termination of breast-feeding, rather than the amount of time since weaning that was associated most closely with altered small intestinal mucosal function'– implying, as in this study, variable C rather than variable D.

However, Goto et al. reported a negative relationship (the early termination of breastfeeding worsened intestinal permeability values), while this study found a positive one. Thus the direction of the association reported in this study is unexpected. Yet the two studies, while both of poor peri-urban communities, are not entirely comparable, given the age of children included in the investigations. The Guatemalan children were 0-11 months old, with 28% having ceased breastfeeding at 6-11 months of age. In the Nepal study, by contrast, children did not stop breast feeding under 1 year of age and breastfeeding was terminated at an average 24 months. In sum, the Guatemalan children were weaned much earlier than the Nepali children, and the timing of weaning events may well differentially affect gut function. The Nepali sample included a large range of ages (0-60 months). This includes both a precarious time of life with the introduction of weaning foods, at 3-6 months to age, and a more settled period after 12-15 months of age, when growth can be expected to stabilise and gut permeability may not further worsen. It is possible that insults to the gut and challenges to the immune system have a different impact on child health at these different ages.

It is well known that breastfeeding gives huge advantages for the infant's health and growth: the milk contains the appropriate amount of lactose as well as proteins, lipids and mineral and is ideally suited for the maintenance of the infant's bodily functions and development. As well as this, the breast milk provides immunity to the newborn baby against infections, as it is much less contaminated with pathogens than bottle feeds which are often prepared using contaminated water in unsanitary environments in developing countries (Sahni and Chandra, 1983). Goto et al. outlined several reasons why breastfeeding should prevent damage to the mucosal function in younger children. Breastfeeding is likely to be associated with a reduced exposure to pathogens, a more rapid recovery from actual infections (due to protective factors in human milk), and fewer nutritional deficiencies (iron and zinc are absorbed more efficiently from breast-milk than from non-milk sources). They also suggested that breastfeeding might affect mucosal function only in younger infants. For example, breast milk contains protective factors including oligosaccharides which are thought to bind with the sites on epithelial cells or with pathogenic bacteria themselves, so reducing bacterial adhesion to the gut. The concentration of oligosaccharides is highest at birth and slowly declines with time. Factors such as this may have an important role in the protection of the infant during the early part of infancy when the gut is so immature.

5.4. CRP and Morbidity

Younger children showed higher CRP values as well as higher morbidity reports. This result indicates the ubiquity of infectious diseases among younger ages in the urban poor communities. A negative relation between CRP and age was also found in young Ghanaian children under 5 years olds (Filteau et al., 1995) and in Samoan youth 4-20 years old (McDade et al., 2000). In the present study, the community Balaju (located on higher riverbank) showed higher CRP levels than the other community sampled. This is difficult to explain. Perhaps the timing of measurement in the community was at the middle of winter, so many diseases, such as respiratory diseases and diarrhoea, should be common among children, therefore the total levels of CRP among children in the community became higher than at the timing of interview in the other community.

Higher values of CRP were associated with child's illness on the day of the interview, rather than illness over the preceding 7 days. The nature of CRP is to react as a 'short-life' acute-phase protein and to remain elevated for 24-72 hours after microbial infections or injury. Thus, there is a strong agreement between high CRP values and maternal reporting of child ill-health in these urban poor communities.

Proxy reporting of child ill-health from mother or guardian is obviously necessary to detect the prevalence of child's morbidity. The maternal reports can obtain the present child ill condition with its duration, episode and severity. Generally, the method is relatively easy and allows for quick collection of information from a large number of people at relatively low cost. Several studies used health interviews to detect the prevalence of illness in the field studies. However, the reporting of morbidity is often challenged about its accuracy and reliability. To minimize the errors of proxy reporting, the recall period is recommended not longer than two weeks and the methods of interview should consist of general and open-ended questions (Kroeger, 1983; Ross and Vaughan, 1986). The cross-cultural study, particularly, should use local interviewers who came from the area as the study population and the 'standardization' of perceived illness should follow the local categorization of illness (Kroeger, 1983). Therefore, the present study used the preceding 7 days for recalling period and a local female assistant for interviewing.

Several researches found maternal morbidity reports to be useful in the field study of child health. In Nepal a field survey in villages showed good agreement between reported morbidity and clinical condition of children: Katz et al. (1998) found good agreements for reported ear infection, measles, diarrhoea and fever.

Using biochemical markers together with morbidity reports, the prevalence of morbidity, particularly diarrhoea, was correlated with higher levels of inflammation markers as well as lower serum protein concentration and poor intestinal permeability (Rousham et al., 1998) and higher acute-phase protein was associated with morbidity (Filteau et al., 1995; McDade et al., 2000; Hautvast et al., 2000). In the study of rural Bangladesh, maternal reports of child ill-health (episodes of fever, diarrhoea and respiratory diseases) were also associated with lower nutritional status showing a clear seasonal change in both morbidity and nutritional status (Rousham and Mascie-Taylor, 1995).

However, maternal reports are limited to clinical illnesses of children, and the amount of under- or over-reporting cannot be estimated in the absence of clinical examinations. For example, Panter-Brick et al. (2001) established the different rural and urban profiles for reported morbidity. In their study, village children reported the lowest morbidity among the groups (squatters, street children and urban middle-class children), however village levels of acute-phase protein were 3-5 times higher than urban groups, which contradicted the reported evidence for illness. They concluded 'it is also likely that villagers significantly under-reported symptoms of ill-health due to a routine experience of illnesses and a low expectation of health care'. The authors reported that reported morbidity, therefore, reflects not only the infection burden, but also different experiences and recognition of ill-health as well as different demands for treatment.

5.5. Anthropometric Status and Biochemical Markers along with Weaning Behaviour

Finally, there was no direct association between gut function and acute-phase protein and anthropometric status per se, but the levels of intestinal permeability and height-for-age in non-breastfeeding children were associated with weaning practice stage 2, the timing of cessation of breastfeeding. Regarding the relationship between weaning practices and infants' growth, McDade and Worthman (1998) mentioned that the benefit of breastfeeding can not be fully understood without considering the complexity of weaning practices in different biosocial settings in both mothers and children. Recently, the World Health Organizations (WHO) re-considered the optimal duration of exclusive breastfeeding in the 54th World Health Assembly in March 2001. In the developing country settings, the advantage of exclusive breastfeeding for 6 months over exclusive breastfeeding for 4 months relates to reducing the risks of infectious morbidity and mortality, especially due to diarrhoeal diseases (WHO, 2001); repeated attacks of diarrhoea for infants contribute toward malnutrition and growth retardation. It relates to food malabsorption, direct nutritional losses and anorexia of child as well as food restriction by mothers (Mahalanabis, 1983).

Results of the relationship between the levels of intestinal permeability and anthropometric status per se do not support the suggestion that gut damage is significantly associated with growth retardation in poor developing countries (Lunn, 2000). However, these negative results could be attributed to several factors. First, this study is cross-sectional rather than longitudinal, which is an important limitation to show child growth per se. Second, the urban poor Nepali children were only mildly stunted, with just 37% of children actually stunted in height. Third, observed intestinal permeability ratios showed a rather large range of raw values, which could not be explained by either morbidity or helminth infestation although they were related to Giardia and to weaning practices. Fourth, the children in this study were relatively old (mean age 45 months), so that the association between anthropometric status and intestinal permeability might not be clearly evident. By contrast, the studies in the Gambia focused on infants 3-15 months old, a time of life when damage to the gut has its most significant effect on growth.

5.6. Conclusion

In conclusion, this study showed significant relationships between the timing of cessation of breastfeeding and two variables, intestinal permeability and anthropometric status (height-for-age), even though there was no direct association between anthropometric status and biochemical markers such as intestinal permeability and acute-phase protein per se. It also found a significant association between Giardia infection and poor intestinal permeability, despite the small number of children affected by protozoa in the sample. Lastly, maternal morbidity reports among the urban poor in Nepal showed a good agreement to measured levels of acute-phase protein in individual children.

These results are limited by the cross-sectional nature of this study in terms of an examination of child growth status which could be explained better in the changing of anthropometric status over time. Furthermore, the children were only mildly stunted beyond the first year of life. Therefore, the association between anthropometric status and biochemical markers might not be clearly found. This study showed the importance of weaning practices as a two-stage process – the onset of supplementation and the cessation of breastfeeding. These two stages may have a differential impact on children's health, in a complex relationship between children's early life stage, the timing of weaning events, and nutritional status of children. Maternal report was useful as a proxy for child morbidity, and CRP is also an especially sensitive infection marker reflecting children's acute illness in urban Nepal.

APPENDIX 1: COMMON PARASITES IN NEPAL

This information about parasites below referenced by Cheesbrough (1987) and Juckett (1995).

Helminths

Ascaris lumbricoides (roundworm)

Ascaris lumbricoides (roundworm) is a very common parasite throughout the world. It is a soil-transmitted helminth via faecal pollution. Contaminated foods and human hands transfer eggs from the polluted soil to the human host. Ascaris produces many eggs which are surrounded by a strong shell and able to stay in the soil for several years resistant to desiccation. Heavy Ascaris infection is common in children aged 3-8 years old, when there is a tendency to get contaminated fingers while playing in open ground. The size of unfertilised egg is about 90 x 45 μm and the surface is a dark green colour with large granules (Figure 1). The larva develops inside the egg, hatching in the small intestine only after being swallowed by the host. The larva penetrates the intestinal wall and transfer to the lung-heart blood circuit where it develops further. When developed, the larvae are swallowed into the stomach again and grow to mature worms (male 15-30cm, female 20-35cm; see picture 3-2: a matured male worm found in a four year-old boy's stool) which then produce eggs to be expelled in the faeces. Egg production occurs about 2 months after infection. The larvae which have migrated to the heart-lung system can cause pneumonia-like symptoms, and coughing similar to attacks of asthma, lasting for 1-2 weeks. The mature worms cause abdominal pain, nausea, diarrhoea and vomiting. For this reason, heavy infection is a cause of malnutrition.

Figure 1. ***Ascaris lumbricaides*** ***(egg)***

Trichuris trichiuria (whipworm)

Trichuris trichiuria (whipworm) is widespread in South East Asia, as well as in the Caribbean and tropical areas of Africa. This infection is often found in association with Ascaris as it is also transmitted by faecal polluted soil. The egg is about 50 x 25μm, has a characteristic barrel shape and is yellow-brown with a colourless boss at each end containing a granular mass in the middle (Figure 2). The larvae hatch in the small intestine and penetrate the villi, then migrate to the large intestine to develop. Worms can live in the host for several years. The matured male has a coiled tail up to 40mm in length while the female is longer, reaching about 50mm. Worms burrow into the intestinal wall, which may be enough to produce mild, chronic blood loss. The female lays eggs passed in faeces. The eggs can remain infective for several months in moist, warm soil. Light infection showed few symptoms in the host, but heavy infection can cause abdominal pain, chronic diarrhoea, ulceration, anaemia and weight loss.

Figure 2. *Trichuris trichiuria* *(egg)*

Hookworm

Hookworm is soil-transmitted by faecal contaminated soil. The larvae in the soil penetrate the skin of the host, especially when the person is walking barefoot on the ground. After penetration, the larvae enter the blood vessels, migrating to the heart-lung circulation to develop. The larvae are then swallowed into the stomach of the host, reaching the small intestine for maturation. The matured worms are 10-15mm in length. These worms attach themselves to the intestinal wall with their mouths and take in mucous and blood, but much of the blood leaking from the wall is lost without being ingested by parasites. The worm takes 5 weeks to produce eggs in faeces after the infection. The egg is 65 x 40μm, colourless with a thin membrane (Figure 3). Heavy hookworm infection causes iron deficiency anaemia or chronic fatigue; a worm sucks about 0.03-0.15ml of blood per day and can stay in the host for 10-15 years.

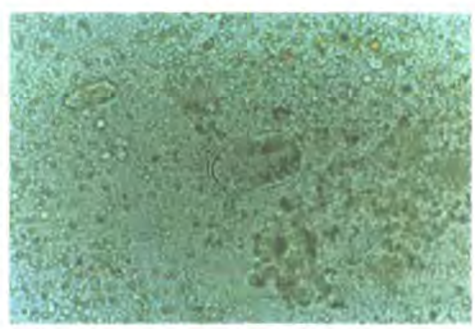


Figure 3. Hookworm (egg)

Protozoas

***Giardia lamblia* (Giardia)**

Giardia is common in the tropics and subtropics areas. Water, food and human fingers can be contaminated by small cysts of Giardia (10 x 6μm) via faecal contaminated soil. The cysts are ingested orally by the host and developed to flagellates in the duodenum and jejunum. The flagellate is also small (12 x 6 μm) and is shaped like a European pear. They attach themselves to the intestine wall and absorb nourishment through their body surfaces. They multiply very fast and only a few cysts are sufficient to develop acute diarrhoea. Giardia can damage the mucosa function of the intestinal wall, which is a cause of maldigestion and malabsorption, especially of carbohydrate, vitamin A and B_{12} (Solomons, 1982; Farthing, 1984).

Entamoeba histolytica

A few types of *Entamoeba histolytica* cause amoebiasis. This infection is common in tropical and subtropical areas but the distribution is related more to poor sanitation than to climate. The cysts of *E. histolytica* in food or water contaminated by faeces are transmitted to the host orally and the cysts then develop in the small intestine. Amoebiasis produces dysentery with blood and mucous in the stool both with little abdominal pain and no fever or vomiting.

APPENDIX 2: RESULTS

Table 1. Number of children in different age groups

Age Groups (month)	Number (%)
0-5.99	15 (7.1)
6-11.99	25 (11.9)
12-23.99	45 (21.4)
24-35.99	38 (18.1)
36-47.99	44 (21.0)
48-60.00	43 (20.5)
Total	210 (100.0)

NB: 0-11.99 month; N=40 (19.0%)

Table 2. Number of children in three different ethnicity groups

Ethnicity (caste)	Tibeto-Burmese	Indo-Aryan (high)	Indo-Aryan (low)
Number (%)	126 (60.0)	50 (23.8)	33 (15.7)

NB: Muslims and Indians were excluded.

Table 3. Levels of intestinal permeability in squatter children

Variables	Number	Mean	Median	SD	Range
L/M ratio*	158	0.32	0.27	0.24	0.04-1.72
Log L/M ratio	158	-1.33	-1.32	0.60	-3.34-0.54

(L: lactulose, M: mannitol, L/M ratio: lactulose/mannitol ratio)

*The distribution departed from normality (Kolmolgorov-Smirnoff test: $p<0.0001$)

Table 4. Levels of lactose and lactose/lactulose ratio

Variables	Number	Mean	Median	SD	Range
Lactose*	168	13.65	5.56	20.34	0.004-3.13
Log lactose	168	1.73	1.71	1.42	-1.87- 4.72
Lactose/lactulose ratio	157**	0.84	0.31	1.52	0.01 -7.84
Log lactose/lactulose ratio	157**	-1.15	-1.15	1.44	-4.28 - 2.49

*The distribution departed from normality (Kolmolgorov-Smirnoff test: $p<0.0001$)

**in 158 lactulose/lactose values, one lactose outliner value was deleted

Table 5. Regression analysis for CRP (low/high)

Independent variables	R^2	B	SE	Sig.
	0.064			
Age (month)		-0.005	0.002	0.014
Location		0.154	0.066	0.021

Independent variables include age (month), sex, location (two sites), ethnicity, and age-ethnicity interaction

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